nature reviews neuroscience

https://doi.org/10.1038/s41583-025-00976-5

Review article

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Dynamic modulation of the blood–brain barrier in the healthy brain

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Abstract

The blood–brain barrier (BBB) performs intricate and dynamic functions that extend far beyond its traditional role as a static protective barrier, playing a pivotal role in maintaining CNS homeostasis. These multifaceted functions are rooted in its specialized architectural and cellular composition. In this Review, we examine the dynamic modulation of BBB function during physiological conditions and the hypothesis that such modulation contributes directly to neural and glial plasticity. We provide an integrated examination of the BBB's diverse cellular components — endothelial cells, pericytes, astrocytes, microglia and vascular smooth muscle cells — across different CNS vascular segments. We discuss how physiological features and states, including circadian rhythms, physical activity, stress and hormonal fluctuations, dynamically alter BBB permeability and signalling, potentially shaping synaptic function, neuronal circuit dynamics and glial responsiveness. Understanding these mechanisms offers new insights into the neurovascular basis of synaptic plasticity and suggests that the BBB may be an under-recognized regulator and modulator of brain adaptability in both health and disease.

Sections

Introduction

Architectural and cellular composition of the BBB

Regulation of molecule transfer across the blood–brain barrier

Modulation of BBB functions in physiological conditions

From BBB modulation to neural functions

Summary and future perspectives

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Introduction

The blood–brain barrier (BBB) is an evolutionarily conserved, complex structural and functional interface that aims to control the diffusion and transport of molecules and communication between the systemic circulation and brain tissue[1–3]. Recent discoveries have broadened our understanding of the roles of the BBB that go beyond its traditional function as a mere protective barrier against toxins and pathogens and have revealed its dynamic regulation in the healthy brain[4,5]. The BBB is increasingly seen as a dynamic interface that adapts its permeability and functionality to various physiological features and states, such as neural activity, circadian rhythms and metabolic changes, influencing overall brain function[6–9].

In this Review, we begin with a detailed examination of the architectural and cellular composition of the mammalian BBB, emphasizing the interactions among endothelial cells, pericytes, astrocytes and other elements of the neurovascular unit (NVU). We continue with more details on transport mechanisms across the BBB and the molecular mediators that govern its functions, with a special focus on the regulatory mechanisms of permeability and transport. Additionally, we explore how the BBB adjusts to changes in hormone levels, metabolism, neural activity and ageing. Finally, we consider the implications of these adaptations for brain function and plasticity and their potential vulnerabilities in various disease states.

Architectural and cellular composition of the BBB

The CNS vasculature consists of distinct segments with diverse cell-type compositions. Arteries and their branching arterioles form the upstream segments in the direction of blood flow, leading to the downstream capillary bed, which drains into venules and veins. The endothelium of brain capillaries, which constitute 85% of the CNS vasculature, and the pericytes that enwrap it comprise the main body of the BBB[3].

The BBB is composed of several cell types (Fig. 1). The main cellular components are endothelial cells, vascular smooth muscle cells (VSMCs) at arteries and arterioles, astrocytes and pericytes at the capillary level. Together with neurons and microglia, these cells form a functional NVU[1]. Recent research in mice, using single-cell RNA sequencing of the cellular milieu comprising the BBB across different vascular segments and brain regions, has revealed transcriptomic heterogeneity within these cellular clusters[10,11]. This complexity has shifted the traditional concept of the NVU as the interface that allows glial cells, neurons and the CNS microvasculature to coordinate homeostatic control and react to injury, towards a more dynamic 'neurovascular complex', characterized by distinct functional modules that coordinate vascular responses to both central and peripheral signals[12].

Endothelial cells

CNS endothelial cells are uniquely adapted to form the foundation of the BBB, responding to a range of signals, including oxygen level[13], temperature[14], pH[15], immune modulators, hormones[16] and mechanical forces[17] that modulate barrier permeability. Unlike peripheral endothelial cells, CNS endothelial cells exhibit specialized molecular signatures, expressing proteins such as the components of tight and adherence junctions that seal the paracellular space, greatly limiting solute flux[18]. Single-cell RNA sequencing in mouse brain tissue has identified distinct endothelial cell populations with transcriptomic profiles varying by location (that is, at arteries, capillaries and veins)[10,11].

At embryonic stages, activation of WNT signalling contributes to the barrier phenotype by inducing tight junction formation[19] and the expression of solute carrier (SLC) transporters such as glucose transporter 1 (GLUT1; also known as SLC2A1)[20,21]. These tight junctions undergo refinement during BBB maturation, enhancing selective permeability[22] (discussed subsequently). In addition, endothelial cells secrete the glycocalyx — a carbohydrate-rich layer composed primarily of proteoglycans, glycoproteins and glycolipids — that coats the luminal surface of brain vessels and functions as a physical and charge barrier, regulates vascular permeability, suppresses transcytosis and modulates inflammation[23] (reviewed elsewhere[24,25]). Endothelial cells also express connexins, which form gap junctions and hemichannels between endothelial cells and between endothelial cells and pericytes. Connexin inhibition increases BBB permeability, highlighting the role of these proteins in maintaining barrier integrity[26,27].

Beyond structural features, the metabolic barrier function of the brain endothelium is increasingly recognized as a contributor to the regulation of transport across the BBB and its unique characteristics. Enzyme-mediated mechanisms contribute to this metabolic barrier function. For example, tissue-nonspecific alkaline phosphatase, which is enriched in brain capillaries, regulates barrier permeability and cytoskeletal organization, with inhibition of tissue-nonspecific alkaline phosphatase transiently increasing paracellular leak[28]. Moreover, γ-glutamyl transpeptidase participates in the γ-glutamyl cycle to generate pyroglutamate, which activates Na^+-dependent amino acid transporters at the abluminal membrane[29]. Additionally, brain endothelial cells express a repertoire of drug-metabolizing enzymes, including the cytochrome P450 enzymes CYP1B1 and CYP2U1, and several aldehyde dehydrogenases, which modulate barrier permeability and drug availability and contribute to the enzymatic restriction of drug entry through the BBB[30,31].

Pericytes and vascular smooth muscle cells

Pericytes and VSMCs are mural cells that envelop the endothelial cells of blood vessels, providing structural support and regulating CNS blood flow. VSMCs, which are separated from endothelial cells by an extracellular matrix (ECM) layer, are found on larger vessels such as arteries and veins, where they regulate vessel diameter and blood flow[32].

Pericytes, which are more prevalent along capillaries, are embedded within the endothelial basement membrane[33]. They are heterogeneous in origin, morphology, protein expression and function, with forebrain pericytes derived from neural crest cells, and brainstem and spinal cord pericytes derived from mesenchymal stem cells (reviewed elsewhere[34]). Developmentally, endothelial cells in capillaries attract pericytes by secreting platelet-derived growth factor B (PDGFB), which binds to PDGF receptor-β (PDGFRβ) on pericytes[35]. Adhesion between pericytes and endothelial cells is promoted by paracrine and autocrine secretion of transforming growth factor-β (TGFβ)[36]. TGFβ, released by both pericytes and endothelial cells, has multiple roles in pericyte function, affecting BBB integrity, metabolism, inflammation and phagocytosis[37–39]. Dysregulated TGFβ signalling can increase BBB permeability[40] (Box 1). Pericytes have key roles in maintaining BBB properties, capillary flow, angiogenesis and inflammatory responses through communication with endothelial via gap and adhesion junctions[41–44]. Ageing-associated reductions in pericyte coverage are associated with increased BBB permeability and reduced cerebral blood flow[45].

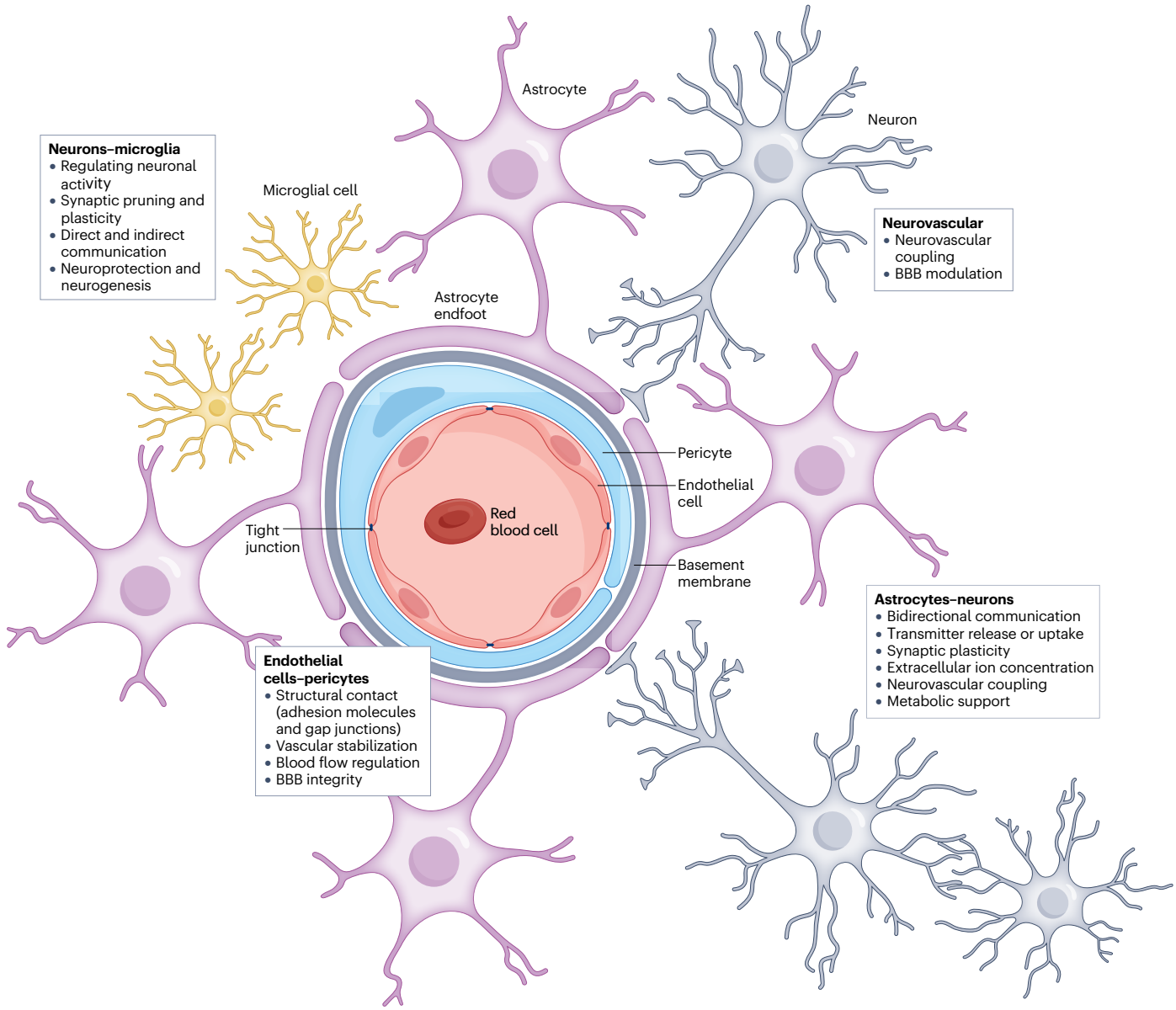

Fig. 1 | Cellular and structural components of the blood–brain barrier and neurovascular unit. The blood–brain barrier (BBB) is a dynamic structure composed of endothelial cells, pericytes, vascular smooth muscle cells and astrocytes, and these cells, together with microglia and neurons, form the neurovascular unit. The various components of the neurovascular unit interact to regulate CNS blood flow, maintain structural integrity and respond to physiological and pathological signals. Endothelial cell tight junctions ensure selective permeability and interact with pericytes to maintain healthy barrier properties and regulate blood flow[238,239]. Interactions between astrocytes and neurons are critical for neurovascular coupling, maintenance of extracellular homeostasis and metabolic support, synaptic activity and plasticity[240–242]. Neuronal activity directly and indirectly affects the brain's microvasculature to increase blood flow and BBB permeability[3,154], whereas interactions between neurons and microglia are important not only in regulating brain inflammation but also for synaptic transmission and plasticity[243].

Astrocytes

Astrocytes, which constitute 20–40% of brain cells, are critical for neurovascular communication and BBB integrity (reviewed elsewhere[46]). Astrocytic endfeet are specialized structures at the tips of astrocyte processes that form a nearly complete covering around blood vessels in the CNS. This arrangement creates a complex interface between the vascular system and the brain parenchyma. Astrocytic endfeet are connected to each other through gap junctions, which primarily comprise connexin 43 (CX43) proteins[47]. These gap junctions allow direct communication and exchange of small molecules between adjacent astrocytes, forming a functional syncytium that can coordinate responses across the NVU[48]. Astrocytes interact with endothelial cells and pericytes through the basal lamina, a specialized ECM structure[49]. Astrocytes maintain BBB properties by secreting factors that influence

Box 1 | The key roles of pericytes in the neurovascular unit

Pericyte-mediated regulation of blood–brain barrier integrity

Pericytes exert their primary impact on endothelial barrier integrity. They act via transforming growth factor-β (TGFβ) signalling and a number of other molecular mediators.

Evidence for the involvement of TGFβ signalling

- TGFβ signalling is essential for the development of blood–brain barrier cell types[246,247].
- TGFβ treatment of pericytes disrupts BBB integrity in vitro[38].
- TGFβ signalling alters the metabolic state of pericytes, shifting energy generation from oxidative phosphorylation towards glycolysis[38].
- TGFβ signalling modulates the inflammatory phenotype of pericytes by affecting the expression of cytokines, chemokines and adhesion molecules (CX3CL1, MCP1 and sVCAM1) in pericytes involved in leukocyte trafficking and microglial function[37].
- The phagocytic ability of pericytes is reduced by TGFβ signalling[37].

Molecular mediators

- The pericyte-expressed gene *Foxf2* regulates barrier permeability[248] and the Notch signalling component RBP-J[249].
- Angiopoietin 1 is secreted from pericytes, binds to TIE2 receptors on endothelial cells and promotes tight junction formation[250].
- Laminin is produced by pericytes and is an important component of the basement membrane.

Pericyte-mediated cerebral blood flow

Evidence indicates that pericytes regulate neurovascular coupling, a close regional relationship between neural activity and blood flow that ensures the supply of energy demands[251]. Pericytes act as contractile cells that locally control capillary diameter to match blood flow with neuronal activity[252]. Moreover, vasoactive molecules such as glutamate, ATP and nitric oxide modulate the contractility of pericytes[253]. K_{ATP} channels, for example, which are inhibited by high intracellular ATP, underlie hyperpolarization of pericytic membrane potential under low ATP conditions, leading to the dilation of capillaries and arterioles during neuronal activity and increasing blood flow[254]. The release of glutamate induces neuronal and/or astrocytic production of vasoactive molecules, such as nitric oxide, that guide the relaxation of pericytes and the dilation of capillaries[255].

Despite these findings, research into pericyte contributions to blood flow modulation under physiological and pathological conditions remains an active area of research as conflicting findings have been reported, probably owing to differences in regional assessment or in experimental preparations. Although some studies support pericytes and capillaries as important regulators of blood flow[253,256], others suggest (i) that arteries and arterioles, which are lined by smooth muscle cells but not capillaries and pericytes, mostly regulate blood flow[20] or (ii) that pericytes do participate in blood flow regulation but with slower kinetics than upstream pre-capillary arterioles[257].

endothelial cell function, including sonic hedgehog[50,51], angiopoietin 1 (ref. 52), WNT growth factors[19,53,54], insulin-like growth factor[55], glial-derived neurotrophic factor (GDNF)[56,57], fibroblast growth factor (FGF)[58] and apolipoprotein E[59] (Box 2).

Aquaporin-4 (AQP4), a transmembrane protein that is concentrated in astrocytic endfeet, facilitates bidirectional water transport across the BBB, allowing water movement both from the blood to the brain and from the brain to the blood[60]. Dysregulated AQP4 expression has been implicated in neuropathologies such as oedema and neuroinflammation, making it a therapeutic target[61,62].

Many studies demonstrating the role of astrocyte-secreted factors in regulating BBB properties have been conducted in cell cultures. However, astrocytes cultured from neonates differ from mature astrocytes in the adult brain, raising questions about the extent to which astrocytes regulate BBB properties in adulthood. Moreover, several factors secreted by cultured astrocytes that have been reported to modulate the BBB in vitro, including sonic hedgehog, glial-derived neurotrophic factor, angiopoietin, FGF2 and vascular endothelial growth factor, are either not detected or found in low concentrations in the mature, uninjured brain[63–65]. However, using tamoxifen-inducible astrocyte ablation in adult mice, a study demonstrated that astrocyte loss leads to the leakage of large serum proteins into the brain[66].

Microglia and parenchymal border macrophages

Microglia are the resident immune cells of the CNS, representing 10–15% of the total cell population[67]. In addition to their role in immune surveillance, microglia are involved in the regulation of synapses and the ECM[68–70], the control of neuronal activity[71] and neurogenesis[72,73]. Although they do not directly maintain BBB integrity, microglia communicate with the cerebral microvasculature from early postnatal stages — migrating along microvessels[74] until their perivascular positions become more stationary in adulthood. Recent findings also suggest a role for microglia in regulating vascular tone and blood flow, both of which indirectly affect BBB permeability (Box 3).

Microglia influence BBB integrity in a context-dependent manner. Early in development, they promote BBB stability via claudin-5 upregulation. Under pathological conditions, activated microglia impair BBB function through phagocytosis of astrocytic endfeet[75], as well as the release of inflammatory cytokines and nitric oxide, which reduce the expression of tight junction proteins and increase BBB permeability[76,77]. Purinergic signalling via P2Y12 receptors supports dynamic microglia–endothelial interactions at sites that lack astrocytic coverage, with potential effects on vascular tone and barrier regulation[78].

Parenchymal border macrophages comprise a resident myeloid cell population that is distinct from microglia and is distributed in the perivascular spaces and leptomeninges. Their exact role in maintaining BBB integrity is not known, but a recent study has revealed their function in regulating cerebrospinal fluid dynamics and BBB integrity under pathological conditions, specifically by regulating cerebrospinal fluid (CSF) influx into the brain interstitial fluid (ISF)[79].

The intricate interplay among endothelial cells, pericytes, astrocytes, microglia, parenchymal border macrophages and neurons highlights the dynamic and complex regulatory mechanisms of the BBB.

Regulation of molecule transfer across the blood–brain barrier

The endothelial cells of the cerebral vasculature possess unique properties that are distinct from those in peripheral tissues, enabling rigorous regulation of molecular traffic between the blood and the brain. These properties help to maintain a stable neural environment and include the absence of fenestrations, low levels of vesicle-mediated transcytosis and high mitochondrial expression to support energy-dependent transport[2]. The cerebral endothelium allows the passage of small lipophilic substances under 400 Da, including gases such as dioxygen and carbon dioxide, while restricting the transfer of hydrophilic molecules. This restriction is primarily enforced through the presence of junctional complexes between endothelial cells and the implementation of specific transport mechanisms, such as saturable transporters, adsorptive endocytosis and specialized vesicular pathways (Fig. 2).

Restricted paracellular traffic

The low paracellular permeability at the BBB is mainly due to tight and adherence junctions between neighbouring endothelial cells. Early electron microscopy studies[80,81] showed that tight junction proteins are integral to the structure of the BBB. Tight junctions are connected to an endothelial cell's actin cytoskeleton via scaffolding proteins of the zonula occludins family (ZO1, ZO2 and ZO3)[2], sealing the inter-endothelial cleft and restricting the paracellular passage of hydrophilic molecules[82]. Key tight junction proteins include occludin and the claudins, each of which has four transmembrane domains and two extracellular loops[83–85]. In mice, occludin (~60–65 kDa protein) has been shown to have a role in intact brain development[86], whereas in human endothelial cell lines it has been shown to have a role in angiogenesis[87]. Yet, although a regulatory role for occludin has been suggested in the formation, maintenance[88] and function of tight junctions[89,90], its direct role in limiting paracellular diffusion is controversial, as knocking down occludin in mice embryonic stem cells had no effect on tight junction morphology and permeability[91].

Claudins (~20–27 kDa) are a group of more than 20 membrane proteins, with claudins 1, 3, 5, 11 and 12 being localized to BBB tight junctions[92]. Claudin-5, in particular, has been identified as critical in regulating endothelial paracellular permeability[93]. Other claudins, such as claudin-3 and claudin-11, have also been suggested to have a role in the functioning of tight junctions[94–96], although to what extent remains controversial. Tight junctions also incorporate junctional adhesion molecules, which are members of the immunoglobulin superfamily and contain two immunoglobulin-like domains. Junctional adhesion molecules have crucial roles in tight junction formation and in regulating leukocyte migration across the BBB into the brain[97,98].

Recently, there has been growing awareness of the existence of tricellular tight junctions (that is, tight junctions at the corners where three endothelial cells meet) at the BBB and blood–retina barrier, formed by the four-transmembrane-protein family that includes tricellulin (~65 kDa) and angulins[99,100]. Despite their exact role in the regulation of paracellular migration still being researched, this subgroup of tight junctions has been shown to contribute to junctional barrier maturation[101] and to be involved in the regulation of T cell entry into the brain[102]. Besides tight junctions, another group of junctional complexes that contributes to the limitation of paracellular diffusion is adherens junctions. These junctions are primarily composed of cadherins and stabilize cell-to-cell

Box 2 | Astrocyte-mediated regulation of blood–brain barrier integrity

Astrocytes, as mediators between neuronal and endothelial cells, contribute to blood–brain barrier (BBB) stability. They secrete factors that promote the integrity of the BBB or increase the barrier's permeability.

Astrocyte-secreted factors that promote BBB integrity

- Sonic hedgehog binds to patched-1 receptors on endothelial cells, ultimately increasing expression of claudin-3, claudin-5, occludin and junction adhesion molecule-A[51]. In addition, sonic hedgehog exhibits anti-inflammatory effects, reducing the expression of tumour necrosis factor, IL-6, IL-1β and intercellular adhesion molecule-1 (ICAM1)[50,51].
- Angiopoetin-1 maintains tight junction integrity by preventing the tyrosine phosphorylation of occludin, ensuring its proper interaction with ZO1[50].
- Activation of WNT–β-catenin signalling pathway in endothelial cells promotes the expression of claudins and occludin, downregulates caveolin-1 expression and reduces immune cell trafficking[19,53,54].
- Insulin-like growth factor-1 contributes to BBB stability by decreasing immune cell trafficking[55]. Glial-derived neurotrophic factor increases the expression of claudin-5, occludin and ZO1[55–57]. FGF proteins — most prominently FGF2 — promote BBB integrity, at least partly, by upregulating occludin and ZO1[58].
- Laminin regulates the differentiation of pericytes and the expression of tight junction proteins[258].

Astrocyte-secreted factors that increase BBB permeability

- Vascular endothelial growth factor signalling increases BBB permeability by downregulating tight junction proteins and upregulating endothelial nitric oxide synthase (eNOS) and nitric oxide production and increases the expression of adhesion molecules (ICAM1 and VCAM1), facilitating leukocyte adhesion and trafficking[259,260].
- Apolipoprotein E (APOE) is produced and secreted by brain astrocytes. Notably, the APOE4 isoform — but not APOE3 — has been linked to increased BBB permeability[59].
- Activation of matrix metalloproteinase-2 (MMP-2) and MMP-9 degrades extracellular matrix components and tight junction proteins[261,262].
- Glia reactivity induces the release of pro-inflammatory cytokines (for example, IL-6 and tumour necrosis factor) and chemokines (for example, CCLX), resulting in barrier weakening — characterized by a decrease in transendothelial electrical resistance — through the STAT3–serpina3n signalling pathway[263].
- Endothelin-1 impairs BBB integrity by promoting the expression of MMPs and vascular endothelial growth factor in endothelial cells, downregulating aquaporin-4 and activating nuclear factor-κB signalling[264].

Box 3 | Neurovascular coupling and the blood–brain barrier

Neurovascular coupling (NVC) refers to the close temporal and spatial relationship between neural activity and cerebral blood flow. NVC ensures that active neurons receive an adequate supply of oxygen and nutrients by triggering localized changes in blood flow. Although NVC and blood–brain barrier (BBB) integrity are distinct concepts, they are interconnected within the neurovascular unit, which comprises neurons, glia and vascular cells that collectively regulate both NVC and BBB function. Astrocytes have a crucial role in NVC by mediating vasodilation through several mechanisms (for example, the release of vasoactive substances)[265]. Astrocytes also contribute to BBB integrity by forming endfeet that ensheath blood vessels and secrete various factors that regulate tight junction proteins[5,266] (see also Box 2). Microglia, while traditionally known for their immune functions, have recently been recognized as important contributors to NVC and BBB regulation. In the mouse brain, microglial processes have shown to create direct contacts with endothelial cells (in arterioles and capillaries), smooth muscle cells, pericytes and astrocytes[267] and to be involved in blood flow regulation and local vascular responses to neuronal activity (that is, NVC)[267,268]. An additional pathway for microglial vasomodulation, specifically vasoconstriction, has been shown in the mouse retina, where the activation of fractalkine–CX3CR1 signalling, which mediates neuron–microglial cell communication, promotes a decrease in vessel diameter via an angiotensin II receptor type-1-dependent mechanism[269]. Evidence suggests that BBB dysfunction is associated with impaired NVC. For instance, in one study[153], recurrent seizures were associated with BBB dysfunction and neurovascular uncoupling both in an in vitro culture model and in vivo.

In addition, BBB disruption has been associated with sustained systemic inflammation, which can further affect NVC[270]. Studies in experimental diabetes have shown higher BBB permeability with downregulation of tight junction proteins[271]. Human studies confirm higher BBB permeability in patients with diabetes[272,273] as well as neurovascular impairment associated with cognitive decline[274,275].

Understanding the complex interplay between NVC and BBB integrity, as well as the roles of astrocytes and microglia in these processes, is crucial for developing targeted therapies for neurovascular disorders and associated cognitive impairments.

interactions. Vascular endothelial cadherin (VE-cadherin) has been reported to mediate calcium-dependent adhesion, interacting with cytoplasmic proteins such as β-catenin and p120-catenin to anchor cadherins to the cytoskeleton and regulate their function[103].

Transcytosis

Transcytosis involves the transport of macromolecules across endothelial cells by clathrin-coated vesicle-dependent and caveolae-dependent pathways[104,105]. This vesicular route includes two transcytosis processes: receptor-mediated transcytosis (RMT) and adsorption-mediated transcytosis (AMT). RMT enables the shuttling of macromolecules — many of which are essential for brain development, function or maintenance — via ligand binding to a receptor on the luminal side and subsequent detachment and release on the abluminal side[106]. One well-known molecule that uses the clathrin–RMT pathway for transport from the blood to the brain is insulin, for which transport is mediated by insulin receptor[107,108]. Another important molecule that undergoes clathrin-mediated transport is the neuropeptide leptin, which is involved in the regulation of appetite and is transported from the blood to the brain by an interaction with the leptin receptor[109,110]. The transferrin receptor and endothelial low-density lipoprotein-related receptor-1 (LRP1) also use the clathrin machinery for the transport of their substrates. Although the former primarily delivers iron transferrin from the blood to the brain[111], LRP1 is thought to mediate the bidirectional transport of the peptide amyloid-β — a 36–43 amino acid peptide that is the main component of amyloid plaques found in the brains of people with Alzheimer disease — facilitating its efflux (clearance) from the brain to the blood[112]. By contrast, the influx of amyloid-β from the blood to the brain appears to be associated with the receptor for advanced glycation end products (RAGE)[112,113]. A second LRP member that participates in vesicular transport is LRP8 (also called apolipoprotein E receptor 2). In the adult brain, its ligand, reelin, is involved in neurogenesis[114], whereas during brain development it regulates neuronal migration and microtubule stability[115,116]. Although BBB-specific studies are scarce, the generation and maintenance of an asymmetric distribution of lipids between the two sides of the cell membrane is a foundational mechanism, achieved using the P4-ATPase flippase complex, which mediates the translocation of phospholipids across the membranes while hydrolysing ATP molecules[117,118].

Unlike RMT, AMT does not require a specific receptor and it involves nonspecific binding of ligands to the endothelial cell membrane. Both clathrin and caveolae facilitate the transcytosis of various cationic peptides and proteins through AMT, characterized by nonspecific, low-affinity interactions between cationic molecules and anionic components of the endothelial cell membrane[119,120]. Caveolae, first described in the 1950s[121,122], are flask-shaped vesicles (50–80 nm) that are smaller than clathrin-coated vesicles (100–150 nm) and that are enriched in phospholipids, sphingolipids, ethanolamine plasmalogens and cholesterol. Formation of caveolae requires the proteins caveolins and cavins, and these vesicles are involved in several processes, including lipid homeostasis, cellular signalling and the transcytosis of small proteins, predominantly from the bloodstream into the brain[123]. A notable example of caveolae-mediated transcytosis is the transport of albumin, a common plasma protein. Albumin interacts with endothelial cell membranes via the albumin-binding protein GP60, facilitating its caveolae-dependent transport across the BBB[124,125]. Other substrates that are transported via caveolae include LDL, which undergoes receptor-mediated transcytosis[126], and amyloid-β, which is internalized through interaction with RAGE and transported via a caveolae-associated mechanism[127].

In the mature, intact BBB, transcytosis is regulated, at least in part, by the secretion of the ECM protein vitronectin from pericytes. Vitronectin binds to the integrin α5 receptor on endothelial cells to inhibit transcytosis[128–130]. The WNT–β-catenin signalling pathway also regulates transcytosis and maintains BBB properties by controlling the transcription of genes such as *MFSD2A* (major facilitator superfamily domain-containing protein 2a)[131]. MFSD2A functions as a lipid flippase, transporting phospholipids such as docosahexaenoic acid, an essential omega-3 fatty acid for the brain, in the form of lysophosphatidylcholine, thereby influencing the lipid composition of

endothelial cell membranes and reducing their suitability for caveolae formation[22,131,132].

Transporter-mediated active passage

Endogenous transporters at the BBB allow the selective influx and efflux of specific nutrients and metabolites. These include SLC transporters and ATP-binding cassette (ABC) transporters. SLCs are encoded by a family of ~400 recognized genes and consist of both influx and efflux transporters[133]. One well-described SLC is GLUT1, a passive uniporter characterized by a high binding affinity for glucose and a lower affinity for other carbohydrates such as mannose and galactose. *GLUT1* mutations[134] have shown to be associated with early life seizures, developmental disorders[135,136] and dysarthria[137].

An additional group of SLCs is the amino acid transporters, which support the delivery of neutral, cationic and acidic amino acids[138]. Trafficking of large and neutral amino acids, such as phenylalanine, tyrosine and tryptophan, is achieved via an exchanger from the L-system amino acids transporter family[139]. Cationic amino acids, such as arginine, are transported using a voltage-sensitive system y+ transporter that is expressed on both luminal and abluminal sides of endothelial cells[140]. One such transporter, SLC7A1 (also known as CAT1), imports arginine from the blood into the brain[141], where it is suggested to be used for the synthesis of endothelial nitric oxide[142]. Although these two groups of amino acid transporters do not require energy, the regulation of amino acids that are naturally found in the neural tissue environment (for example, the acidic amino acids glutamate and aspartate) is controlled by active efflux transporters that are expressed exclusively at the abluminal side of endothelial cells, which move amino acids against their concentration gradient from the brain to the blood[138]. An example of such a transporter is the voltage-sensitive excitatory

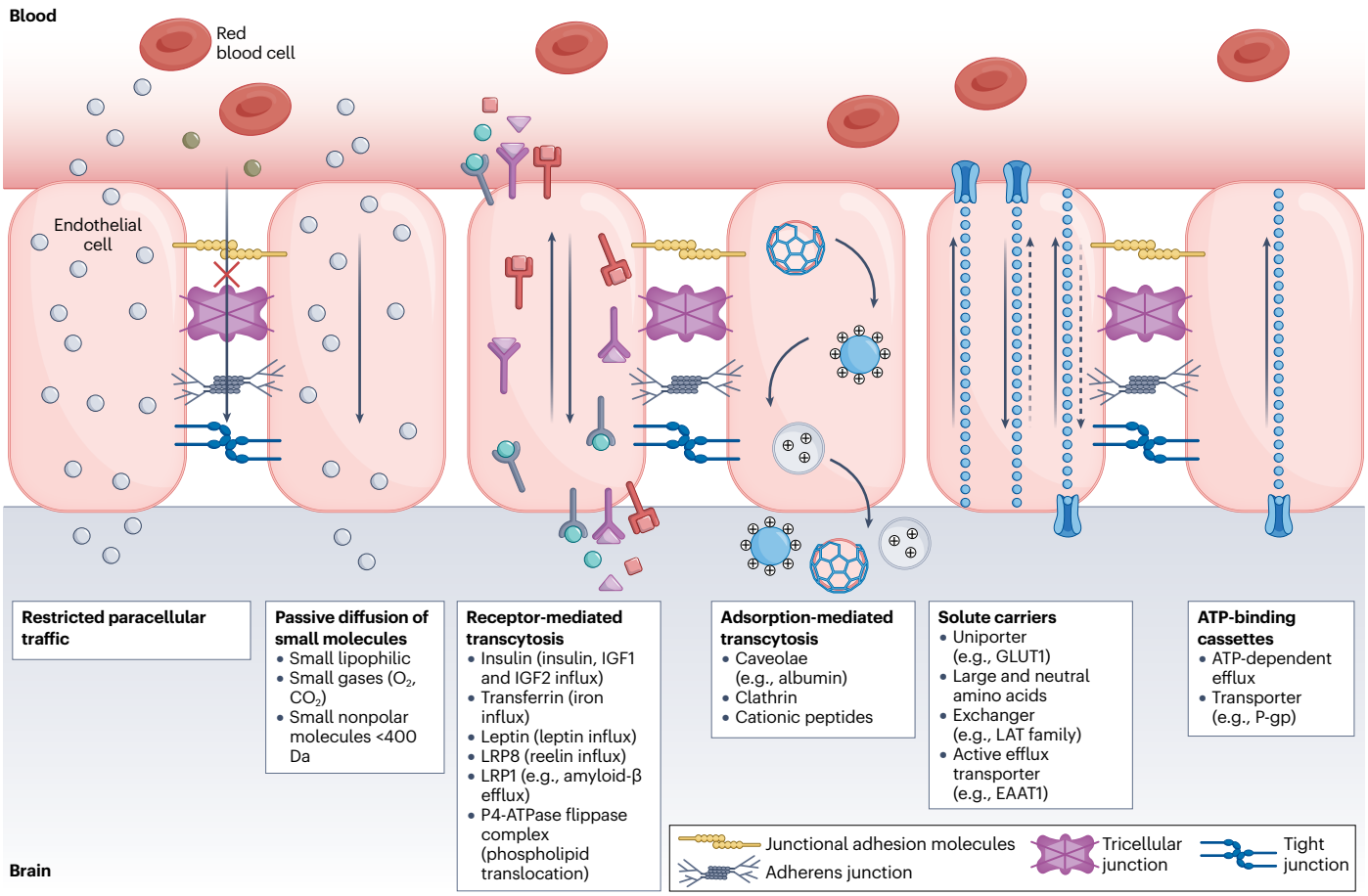

Fig. 2 | Transport mechanisms across the blood–brain barrier. The bidirectional movement of hydrophilic molecules between the bloodstream and the brain is tightly regulated by the blood–brain barrier via paracellular and transcellular pathways. Tight and adherens junction proteins, tricellular junctions and junctional adhesion molecules between endothelial cells restrict paracellular transport while allowing small lipophilic molecules to diffuse freely through the endothelial cells[2,82]. Receptor-mediated transcytosis enables selective influx (for example, insulin and iron-transferrin)[108] and efflux (for example, amyloid-β)[244] of specific molecules. Adsorptive-mediated transcytosis, mediated by clathrin and caveola, facilitates a nonspecific transport of cationic peptides and proteins via electrostatic interactions with endothelial cells[127]. Transporter-mediated machineries include: solute carrier transporters, which mediate the import of nutrients (for example, glucose and amino acids) from the blood to the brain; an energy-dependent efflux system that exports neurotransmitters (for example, glutamate and aspartate) from the brain to the blood[143], with each export step coupled to an influx of three Na^+ ions and the efflux of one K^+ ion; and ATP-binding cassette transporters, such as P-glycoprotein (P-gp), which clear metabolites and xenobiotics from the brain into the bloodstream[150]. EAAT1, excitatory acidic amino acid transporter 1; GLUT1, glucose transporter 1; IGF, insulin-like growth factor; LAT, L-system amino acids transporter; LRP1, low-density lipoprotein-related receptor-1.

acidic amino acid transporter 1 (EAAT1; also known as SLC1A3), which mediates the efflux of glutamate and aspartate. It gains the energy to do so by co-transporting three Na^+ and one H^+ and by counter-transporting one K^+ (ref. 143). Impaired glutamate uptake owing to a mutation in *EAAT1* is likely to play a major role in the development of seizures, hemiplegia and episodic ataxia[144].

ABC transporters are a family of active, ATP-dependent systems involved in the efflux and clearance of brain metabolites into the bloodstream. Forty-eight ABC transporter genes, encoding 44 membrane transporters, have been identified in humans[145,146], and although they are predominantly expressed on the luminal side of brain endothelial cells, they are also expressed in neurons and astrocytes, where they play a key role in cholesterol regulation[147]. One notable ABC transporter is P-glycoprotein (P-gp; ~170 kDa), which is encoded by *ABCB1* (ref. 148). P-gp is crucial for clearing amyloid-β from the brain[149], yet its overexpression in various tumours, including glioblastoma, restricts the entry of anticancer drugs into the brain. This has made P-gp a target for the development of inhibitors intended to enhance drug distribution within the brain[150]. However, clinical application of P-gp inhibitors has been limited by low efficacy and systemic toxicity, particularly owing to high ABC transporter expression in the liver[151].

Modulation of BBB functions in physiological conditions

BBB function and maintenance rely heavily on signalling from the NVU to the endothelial cells, including via WNT ligands, TGFβ signalling and ECM factors (reviewed elsewhere[3]). Contrary to traditional views, the BBB is highly dynamic, modulating its properties in response to various physiological stimuli. Recent studies highlight the adaptability of the BBB to metabolic, circadian and neural demands through electrophysiological, molecular, cellular and systemic mechanisms, positioning the BBB as an active regulator of brain homeostasis (Table 1).

Neuronal activity

Several studies indicate that changes in neuronal firing can influence BBB permeability. Increased neuronal activity raises local metabolic demands, meaning that enhanced transport is needed across the BBB to supply essential nutrients such as glucose and oxygen. Although most

Table 1 | Physiological influences on the blood–brain barrier

Physiological condition	BBB modulation	Refs.
Neuronal activity	Increasing neuronal stimulation in the cortex leads to an increase in caveolae-mediated transcytosis of albumin and TGFβ signalling	154
	Transcranial magnetic stimulation leads to local increases in BBB permeability to gadolinium-based contrast agents	155
	Chemogenetic stimulation regulates BBB efflux by altering endothelial cell ABC transporter expression and function, as well as altering the expression of circadian clock genes involved in efflux	159
Circadian rhythms	Diurnal oscillations in endothelial transporters such as P-glycoprotein ABCB1 result in diurnal variations of efflux across the BBB, with peak levels during the active phase	160
	The clock gene *Bmal1* regulates pericyte function	164
	The clock gene *Per2* and melatonin regulate water flux through BBB AQP4 channels	165–167
	The tight junction proteins occludin, claudin-1, claudin-5 and ZO2 exhibit daily oscillations in expression levels	171
Physical activity and exercise	Regular moderate exercise improves barrier integrity in rodents and humans	174
	High-intensity exercise leads to a transient increase in BBB permeability	175
	Aerobic training in ageing and injury models upregulates tight junction protein levels and boosts pericyte coverage	176
	Environmental enrichment and voluntary running prevent stress-induced BBB disruption via FGF2 signalling, preserving claudin-5	178[f]
	Aerobic exercise raises serum BDNF and lowers S100B	6
Metabolism	Fasting upregulates expression of the endothelial MCT1 transporter, leading to increased ketone uptake	179
	Insulin signalling leads to upregulation of the tight junction proteins claudin-5 and occludin, modulates amyloid-β and insulin carrier transport and affects endocytosis and efflux profiles	180–182
Physical and mental stress	Acute stress leads to a transient decrease in occludin, ZO1 and claudin-5	9,186
	Transient stress leads to glucocorticoid-mediated upregulation of claudin-5, occludin and VE-cadherin	187
	Chronic stress can lead to persistent BBB opening, mediated by a decrease in claudin-5, an increase in transcellular transport and pericyte loss	188,236
Ageing	Glycocalyx dysregulation, increased transcellular transport, astrocyte senescence, loss of pericyte coverage, and elevated oxidative stress, TGFβ signalling and neuroinflammation drive a progressive increase in BBB permeability	7,191–194, 237
Hormones	17β-Oestradiol enhances BBB integrity by modulating endothelial tight junction proteins and transporters	196,197
	Testosterone modulates BBB function via regulation of tight junction architecture and expression of claudin-5 and ZO1	198
	Progesterone reduces injury-induced BBB disruption, enhances tight junction function and promotes neural regeneration by increasing the circulation of endothelial progenitor cells	199

ABC, ATP-binding cassette; AQP4, aquaporin-4; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; FGF2, fibroblast growth factor 2; MCT1, monocarboxylate transporter 1; TGFβ, transforming growth factor-β.

reports focus on pathological neuronal activation, such as seizures and spreading depolarizations, which increase BBB permeability to serum proteins, probably via caveolin-1-dependent transcytosis[152,153], recent research shows that endothelial permeability can also be modulated by non-pathological repetitive stimulation of cortical neurons[154]. Functional MRI studies in humans corroborate these findings, showing that low-frequency activation of cortical neurons by transcranial magnetic stimulation[155] or repetitive motor activity[154] leads to local increases in BBB permeability to gadolinium-based contrast agents. The effects of neuronal activity on BBB properties are primarily mediated by glutamate acting through NMDA-type receptors (NMDARs), involving TGFβ signalling[154,156]. AMPA-type and metabotropic glutamate receptors have also been implicated in modulation of BBB permeability in in vitro preparations and under ischaemic conditions[157,158]. Neuronal activity induced by chemogenetics was shown to regulate BBB efflux by altering endothelial cell ABC transporter expression and function, as well as altering the expression of circadian clock genes involved in efflux[159]. Circadian clock genes (for example, *CLOCK*, *BMAL1*, *PER* and *CRY*) form part of the molecular machinery that drives the body's ~24-h cycles in gene expression. These genes help to coordinate daily physiological rhythms, including those relevant to the BBB, by regulating processes such as transporter function and metabolic activity in a time-of-day-dependent manner (discussed subsequently).

Circadian rhythms

Circadian rhythms substantially affect BBB function. The expression of several key transporters and enzymes at the BBB exhibits diurnal patterns, modulating substance entry and clearance according to the time of day. For example, within the BBB endothelium of flies and mammals, there are diurnal variations in transporters such as P-gp, resulting in diurnal variations in efflux across the BBB with levels being highest during the active phase and lowest during the resting phase[160]. Several studies have shown in flies, rodents and humans that the concentrations of molecules in the CNS undergo circadian oscillations, probably because of their rhythmic CNS influx or efflux. Examples include inflammatory cytokines (IL-6 and TNF)[161] and leptin — a hormone primarily produced by adipose tissue that has a crucial role in regulating energy balance and body weight by signalling satiety to the brain. A recent study in mice found a diurnal variation in leptin transport across the BBB, with the fastest influx of leptin into the brain occurring in the evening[162]. Another example is amyloid-β, whose dynamics have been shown to be regulated by the circadian clock in a mouse model of β-amyloidosis[163]. Disrupting circadian clock function in mice either globally or locally in the brain by deleting *Bmal1* caused disruption of daily hippocampal interstitial fluid amyloid-β oscillations and accelerated amyloid plaque accumulation, suggesting that circadian rhythms influence amyloid-β dynamics and plaque formation[163]. *Bmal1* also has a vital role in maintaining BBB integrity by regulating several aspects of pericyte function. BMAL1 upregulates the transcription of PDGFRβ in pericytes, which is essential for pericyte recruitment, proliferation, migration and attachment to blood vessels. *Bmal1* deletion leads to age-dependent loss of pericyte coverage of brain blood vessels and BBB permeability[164]. In human primary pericytes, silencing *BMAL1* disrupts angiogenesis and vessel maturation, as well as endothelial cell circadian rhythms, probably owing to an alteration in lactate transportation[165].

The mechanisms that underlie circadian alterations in brain concentrations of metabolites at the BBB are not entirely understood. The circadian clock seems to influence the expression or activity of endocytic machinery components, although current evidence suggests limited transcriptional regulation of BBB-specific genes by the clock[160]. A few studies have suggested a role for circadian-dependent changes in intracellular Mg^{2+} ($[Mg^{2+}]_i$) in BBB properties. In *Drosophila*, the BBB consists of two main glial cell layers: the outer perineurial glia and the inner subperineurial glia. The circadian clock in perineurial glia cells regulates $[Mg^{2+}]_i$ through cyclically expressed gap junctions. During the day, high $[Mg^{2+}]_i$ promotes the activity of efflux transporters (P-gp-like transporters in *Drosophila*), reducing the permeability of xenobiotics across the BBB. At night, gap junctions between perineurial glial and subperineurial glial cells increase, allowing perineurial glial cells to act as an Mg^{2+} sink and thereby lowering $[Mg^{2+}]_i$ in subperineurial glia cells. This decrease in $[Mg^{2+}]_i$ reduces efflux transporter activity[166]. Similarly, in microvascular endothelial cell lines from mice and humans, the cycling of xenobiotic efflux is associated with cycling of $[Mg^{2+}]_i$ caused by transcriptional regulation of transient receptor potential melastatin 7 — a protein that functions as both an ion channel and a kinase[160]. Changes in water flux through the BBB and within the brain's interstitial space can also be subject to circadian rhythms owing to alterations in the polarization, expression and function of AQP4 (ref. 167). The core circadian clock genes, particularly *Per2*, play a role in regulating AQP4 polarization[168]. Post-translational modifications, such as AQP4 phosphorylation, are also involved by affecting AQP4 localization and function[169,170]. Melatonin, a hormone that is closely associated with circadian rhythms, has also been shown to influence AQP4 expression and polarization, potentially through modulation of these clock genes[168].

Proteins such as dynamin and RAB11 enhance endocytosis at the BBB preferentially during sleep[171,172]. In addition, tight junction proteins such as occludin, claudin-1, claudin-5 and ZO2 undergo daily oscillations in expression, influencing barrier permeability[171]. Together with changes in transporter activity and water exchange, these daily BBB function oscillations ensure efficient nutrient influx and waste clearance, adapting to the fluctuating demands of the neural environment. It has been suggested that age-related disruptions in these circadian rhythms could lead to BBB dysfunction and contribute to neurodegenerative diseases[173].

Physical activity and exercise

There are several studies providing some evidence and the theoretical framework to connect physical activity and BBB function, but the full picture is yet to be uncovered. Studies in mice suggest that moderate, regular exercise may have protective effects on the BBB[174]. By contrast, small-cohort human studies have reported that intense exercise is associated with an elevation in the level of serum S100B, suggesting that exercise is linked to increased BBB permeability[175]. The mechanisms underlying these effects are not yet clear but are hypothesized to involve mediators that are modulated during physical activity, including free radicals, neuroinflammatory signalling, systemic inflammation, neurotrophic factors and renin–angiotensin hormonal signalling[176]. For example, aerobic exercise in rodent models of ageing and injury was shown to decrease BBB permeability by upregulating the expression of tight junction proteins and increasing coverage by pericytes[6]. In women aged 65–74 years (but not 75 years and older), aerobic exercise leads to increased serum brain-derived neurotrophic factor and decreased levels of serum S100B, indicative of increased BBB permeability[177]. Moreover, in mice, environmental enrichment and voluntary physical exercise protect the BBB from stress-induced alterations; specifically, they enhance FGF2 signalling and prevent the loss of the tight junction protein claudin-5 (ref. 178).

Metabolic states

BBB permeability also responds to metabolic changes, such as fasting and feeding cycles. In fasting states, the brain relies more on ketone bodies for energy. This prompts the BBB to upregulate transporters such as monocarboxylate transporter 1, through a mechanism involving selective activation of peroxisome proliferator-activated receptor δ (PPAR-δ), in brain endothelial cells, ensuring adequate energy substrate delivery during low glucose availability[179]. Conversely, during feeding, insulin signalling influences BBB properties, increasing tight junction integrity, modulating the transport of substances such as amyloid-β across the BBB and regulating its own blood-to-brain transport through a saturable, regulated process[180]. RNA sequencing of polarized human cerebral microvascular endothelial cell monolayers revealed insulin-induced upregulation of transcripts involved in biological processes that protect BBB functions, such as vascular development regulation and actin cytoskeleton reorganization, and downregulation of transcripts linked to inflammation-related pathways[181]. Moreover, one study[182] showed that endothelium-specific insulin receptor knockout mice exhibited a reduction in tight junction protein expression and an increase in BBB permeability in specific brain regions. Collectively, these findings highlight the importance of insulin signalling in maintaining BBB integrity through multiple mechanisms. In line with these findings discussed earlier, systemic metabolic conditions, such as obesity, insulin resistance and diabetes, can impair BBB function by inducing oxidative stress, inflammation and tight junction alterations, ultimately compromising barrier integrity and increasing permeability[183–185].

Physical and mental stress

Both physical and mental stress can modulate BBB function, and the effects are region-dependent, cell-type-dependent and time-dependent. A single episode of stress can trigger a reversible opening of the BBB that lasts minutes to a few hours, most prominently in hippocampus, prefrontal cortex and hypothalamus[9,186], whereas acute glucocorticoid pulses (cortisol/corticosterone) in vivo or in vitro were reported to transiently tighten the barrier by upregulating claudin-5, occludin and VE-cadherin[187]. In chronic stress, by contrast, sustained high cortisol, oxidative stress, nuclear factor (NF-κB) activation and MMP-9 release lead to persistent BBB opening. In mouse models of chronic social defeat, this is marked by selective loss of claudin-5 in mood-regulating nuclei, heightened transcellular vesicle traffic and pericyte loss and contributes to neuroinflammation, post-traumatic complications and mood disorders[188–191].

Ageing

Ageing is associated with a progressive increase in BBB permeability, driven by glycocalyx dysregulation, increased transcellular transport, astrocyte senescence, loss of pericyte coverage and elevated oxidative stress and inflammation[7,40,43,192–194]. Age-related BBB dysfunction has been shown to lead to neuroinflammation and cognitive impairment with blood-derived albumin leakage into the brain parenchyma, triggering astrocytic activation, massive inflammatory signalling, synaptic dysfunction and excitation-inhibition imbalance[8,40].

Hormonal regulation of BBB

Hormones, including metabolic regulators, sex hormones and stress-responsive hormones, dynamically influence BBB function, modulating its permeability under physiological and pathological conditions. For example, as mentioned earlier, insulin signalling in endothelial cells has a role in maintaining BBB integrity.

Oestrogen, particularly 17β-oestradiol, enhances BBB integrity by modulating tight junction proteins and inhibiting the activation of NF-κB-dependent matrix metalloproteinase (MMP) genes, thereby reducing MMP gene transcription and mitigating tight-junction disruption[195,196]. 17β-Oestradiol also upregulates the activity and expression of Na^+/K^+-ATPase (NKAT), a key protein involved in maintaining ionic homeostasis at the BBB[197].

Testosterone similarly contributes to maintenance of BBB integrity. Studies indicate that testosterone deficiency leads to an increase in BBB permeability by disrupting tight junction architecture and decreasing the levels of claudin-5 and ZO1. Testosterone supplementation has been shown to restore tight junction integrity and BBB function in such cases[198].

Progesterone is another sex hormone with neuroprotective effects on the BBB, particularly following brain injury. It reduces inflammation-induced BBB disruption, enhances tight junction function and promotes neural regeneration by increasing the circulation of endothelial progenitor cells[199].

Less is known about the effects of gonadotropins on BBB properties. The abundant expression of receptors for human chorionic gonadotropin and luteinizing hormone in brain endothelial cells[200], and their increased serum levels with ageing, during which BBB integrity is often compromised, has raised the hypothesis that gonadotropins regulate BBB permeability. Supporting evidence comes from experiments showing that ovariectomy in young mice results in increased BBB permeability, accompanied by changes in the expression and distribution of CX43 (ref. 201). Further studies are needed to delineate the direct effects of sex hormones on BBB integrity and their roles in physiology and pathology.

In this regard, recent studies suggest that pregnancy-induced changes in BBB properties may be linked to alterations in brain structure, particularly in areas associated with social cognition[202]. The overall effect of pregnancy on BBB properties remains incompletely understood. The modulation of BBB properties during pregnancy has been linked to the remodelling of penetrating arterioles, increased capillary density and increased cerebral blood flow[203]. These vascular adaptations are associated with activation of PPARγ, a transcription factor that is highly expressed during pregnancy[203]. Conversely, elevated levels of progesterone and oestrogen during pregnancy may exert protective effects, balancing these vascular changes. In primary human colon tissues in vitro, progesterone increased *trans*-epithelial electrical resistance by upregulating occludin and inhibiting NF-κB-driven inflammation[204]. In rat models of traumatic brain injury, similar protective effects were observed at the BBB, where progesterone reduced inflammation, brain oedema and BBB permeability, ultimately improving neurological outcomes[205].

Early evidence also suggests that pregnancy-related changes in the BBB may facilitate brain adaptations essential for motherhood. However, the extent to which these alterations overlap with BBB changes and how BBB modulation during pregnancy relates to physiological pregnancy-related neuronal plasticity remains to be fully elucidated. Nevertheless, BBB vulnerability during pregnancy appears to contribute to neurological complications in pathologies such as preeclampsia and eclampsia[206].

From BBB modulation to neural functions

Our understanding of how BBB modulation influences neuronal activity is limited (Fig. 3). The downregulation of tight junction proteins, as reported during certain circadian phases or in specific conditions

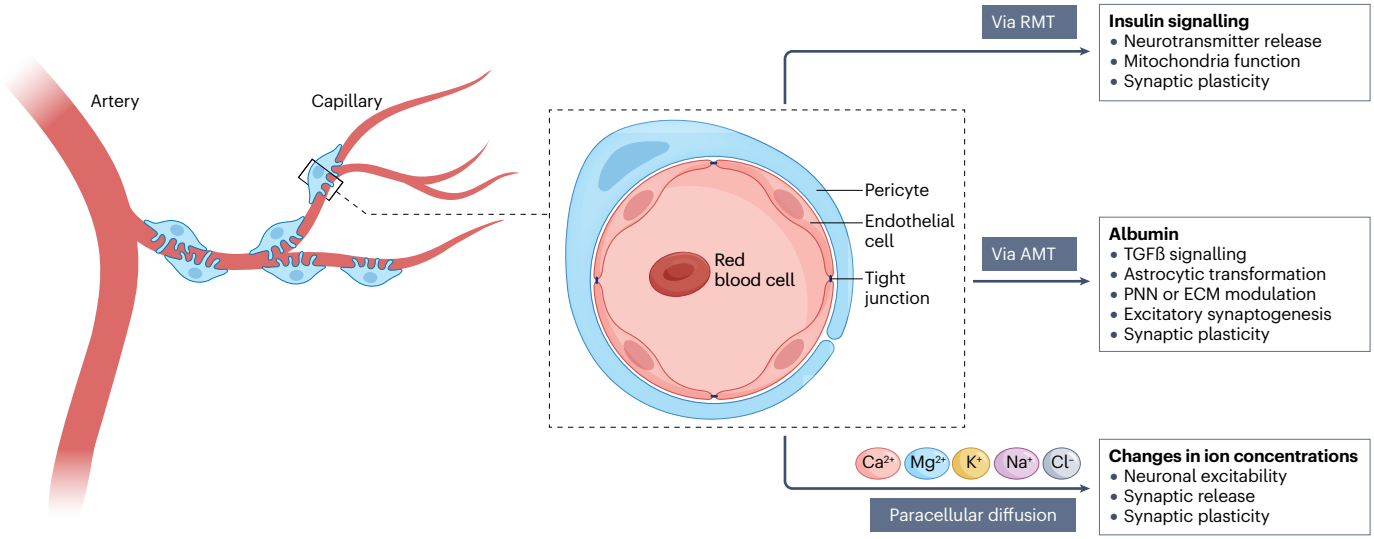

Fig. 3 | Modulation of blood–brain barrier properties underlie alterations in neuronal functions. Alterations in tight junction properties are expected to change brain ion concentrations and result in enhanced excitability, synaptic release and postsynaptic firing. Serum albumin has been shown to cross the blood–brain barrier (BBB) under some physiological conditions[154], leading to alterations in the properties of astrocytes and activity-dependent increases in the concentrations of extracellular K^+ and glutamate, excitatory synaptogenesis and plasticity[154,211,212,245]. Insulin is another example of a signalling molecule that upon BBB modulation alters the mitochondrial stress response, synaptic release and plasticity[217,220]. AMT, adsorption-mediated transcytosis; ECM, extracellular matrix; PNN, perineuronal net; RMT, receptor-mediated transport; TGFβ, transforming growth factor-β.

such as pregnancy, alters ionic concentrations in the ISF, reducing the ionic gradient between the brain and serum. For instance, the ISF maintains $[K^+]$ at 2.5–2.9 mM, whereas the plasma levels of this ion are 3–5 mM. The plasma also has a higher $[Ca^{2+}]$ and $[Mg^{2+}]$ than the ISF. Changes in BBB paracellular permeability can therefore influence ISF ion levels, affecting neuronal resting potential, excitability, synaptic activity and synchronicity[207], and may therefore influence neuronal activity and synaptic plasticity during intense sensory–motor activities, as recently suggested in rodent studies[154], as well as in pathology.

In addition to paracellular effects, NKAT in brain endothelial cells regulates extracellular ion balance and neural excitability, potentially modulated by noradrenergic input from the locus coeruleus[208]. Impaired NKAT function has been postulated to contribute to migraine[209] and stroke[210] pathophysiology.

A path by which BBB modulation can influence neural plasticity is through mechanisms involving extravasated albumin and the subsequent activation of TGFβ signalling. Albumin activates astrocytic TGFβ pathways, leading to homosynaptic and heterosynaptic plasticity, increased long-term potentiation and altered neural circuit function[211]. Additionally, albumin-triggered TGFβ signalling drives excitatory synaptogenesis, contributing to network plasticity[212]. BBB permeability also induces ECM remodelling, particularly affecting perineuronal nets[213]. Together, these changes, induced by BBB modulation, may be critical for the regulation of synaptic stabilization and plasticity[40,188]. Future studies will determine under what conditions these changes contribute to reorganization of neuronal networks under physiological conditions, promote repair or lead to maladaptive outcomes after brain injury.

Another example is insulin's role in brain function. In addition to insulin's effect on brain endothelial cells, insulin receptors are distributed throughout the NVU, particularly in the lateral septum, amygdala, subiculum, hippocampal CA1 region, mammillary body and arcuate nucleus[214]. Although direct experimental evidence is limited, modulation of insulin transport across the BBB via caveolin-mediated or clathrin-mediated mechanisms is expected to increase insulin levels in the brain[108,215]. Insulin signalling affects synaptic plasticity, neurotransmitter release and dendritic spine density, influencing neurotransmitter systems including NMDARs and GABA receptors and altering the function of specific neuronal populations such as agouti-related protein and dopamine neurons[216–218]. Insulin also affects brain glucose metabolism[219], the mitochondrial stress response (in vitro and in mice in vivo)[220] and the neural response to injury[215,221], playing roles in cognitive functions such as memory and learning and mood regulation[222–224].

To what extent the fluctuations in the levels of insulin affect BBB properties, brain insulin levels and, directly or indirectly, neuronal networks is still not entirely clear. However, the existing data support the notion that insulin resistance can be linked to alterations in brain network connectivity[225] and plasticity[226] and therefore may be associated with mood disorders and cognitive decline[227]. Indeed, recent imaging studies in patients with bipolar disorder confirm that insulin resistance is associated with increased BBB permeability, severe depression and lower cognition[228].

Summary and future perspectives

Most research on the BBB focuses on its characteristics under different disease conditions and its role in the pathogenesis of common brain disorders. Recognizing its key role in disease pathology has led to studies on mechanisms underlying BBB modulation in brain disorders, such as the role of neuronal depolarization and the associated massive release of glutamate (reviewed elsewhere[229]). The relevance of BBB permeability changes to neurological disorders has spurred

the search for serum biomarkers reflecting permeability changes and imaging methods to quantitatively measure BBB integrity. As often happens in medical sciences, studying disease mechanisms sheds light on normal physiological processes. In the case of the BBB, understanding the processes that modulate its integrity has led to the recognition that signalling mechanisms previously thought to be unique to disease conditions might be an extreme case of more subtle signalling pathways that modulate BBB function under physiological conditions. This has important implications for the function of the NVU, neuronal network activity and synaptic plasticity.

Although our understanding of the detailed role of BBB dysfunction in diseases has advanced, leading to new diagnostics and therapeutic targets, a large gap remains in our understanding of the mechanisms underlying and role of BBB modulation in physiological conditions. For instance, although there are data suggesting that BBB properties differ among brain regions[230], the details and functional significance of regional differential expression of BBB-related genes are not fully understood. Future studies are needed to explore the detailed differences in BBB properties between brain regions and under different physiological conditions, such as sleep and awake states, metabolic states, pregnancy, stress and physical exercise, and their roles in short-term and long-term modifications of NVU network activity, memory and cognitive capacities.

Recent research has also focused on the glymphatic system, an AQP4-facilitated exchange of CSF with ISF, which is thought to play a crucial role in clearing protein species such as amyloid-β and tau, which accumulate in the brains of patients with neurodegenerative diseases[231–233]. Similar to the modulation of BBB during the circadian cycle, the glymphatic system changes its properties during sleep, enhancing the exchange rate of CSF with ISF and accelerating waste removal. It is worth noting that the glymphatic hypothesis remains controversial, with critiques highlighting that although periarterial solute movement is supported, there is insufficient evidence for bulk flow through perivenous routes and that diffusion may predominate over convective transport in the interstitium[234]. Regardless, although both systems heavily rely on AQP4 expression and localization, the interactions between glymphatic system functions and BBB properties remain unclear[235]. It is also not clear how modulation of BBB properties, as described in this Review, and the transport of proteins into specific brain regions affect the exchange rate through the glymphatic system.

In conclusion, the dynamic nature of the BBB as explored in this Review underscores its critical role not just as a defensive barrier but also as a regulator of the internal environment and plasticity of the CNS in response to physiological stimuli. The multifaceted interactions between the cellular constituents of the BBB and their impact on its permeability reveal a complex system that is crucial for maintaining brain homeostasis and responding to both physiological needs and pathological challenges. As we continue to explore its capabilities and limitations, the future of neurology and neuroscience stands to gain from innovations that harness the insights from these studies, potentially revolutionizing how we approach, diagnose and treat brain disorders.

Published online: 15 October 2025

References

1. Serlin, Y., Shelef, I., Knyazer, B. & Friedman, A. Anatomy and physiology of the blood–brain barrier. *Semin. Cell Dev. Biol.* **38**, 2–6 (2015).
2. Abbott, N. J., Rönnbäck, L. & Hansson, E. Astrocyte–endothelial interactions at the blood–brain barrier. *Nat. Rev. Neurosci.* **7**, 41–53 (2006).
3. Kaplan, L., Chow, B. W. & Gu, C. Neuronal regulation of the blood–brain barrier and neurovascular coupling. *Nat. Rev. Neurosci.* **21**, 416–432 (2020).
4. Keaney, J. & Campbell, M. The dynamic blood–brain barrier. *FEBS J.* **282**, 4067–4079 (2015).
5. Pocit, A., Pivorinas, A. & Verkhratsky, A. Astrocytes dynamically regulate the blood–brain barrier in the healthy brain. *Neural Regen. Res.* **19**, 709–710 (2024).
6. Cho, S. Y. & Roh, H. T. Effects of exercise training on neurotrophic factors and blood–brain barrier permeability in young-old and old-old women. *Int. J. Environ. Res. Public Health* **19**, 16896 (2022).
7. Knopp, R. C., Erickson, M. A., Rhea, E. M., Reed, M. J. & Banks, W. A. Cellular senescence and the blood–brain barrier: implications for aging and age-related diseases. *Exp. Biol. Med.* **248**, 399–411 (2023).
8. Milikovsky, D. Z. et al. Paroxysmal slow cortical activity in Alzheimer's disease and epilepsy is associated with blood–brain barrier dysfunction. *Sci. Transl. Med.* **11**, eaaw8954 (2019).
9. Friedman, A. et al. Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. *Nat. Med.* **2**, 1382–1385 (1996).
10. Saunders, A. et al. Molecular diversity and specializations among the cells of the adult mouse brain. *Cell* **174**, 1015–1030.e16 (2018).
11. Vanlandewijck, M. et al. A molecular atlas of cell types and zonation in the brain vasculature. *Nature* **554**, 475–480 (2018).
12. Schaeffer, S. & Iadecola, C. Revisiting the neurovascular unit. *Nat. Neurosci.* **24**, 1198 (2021).
13. Schoch, H. J., Fischer, S. & Marti, H. H. Hypoxia-induced vascular endothelial growth factor expression causes vascular leakage in the brain. *Brain* **125**, 2549–2557 (2002).
14. Kiyatkin, E. A. & Sharma, H. S. Permeability of the blood–brain barrier depends on brain temperature. *Neuroscience* **161**, 926–939 (2009).
15. Oldendorf, W., Braun, L. & Cornford, E. pH dependence of blood–brain barrier permeability to lactate and nicotine. *Stroke* **10**, 577–581 (1979).
16. Hampl, R., Bičíková, M. & Sosvorová, L. Hormones and the blood–brain barrier. *Horm. Mol. Biol. Clin. Investig.* **21**, 159–164 (2015).
17. Garcia-Polite, F. et al. Pulsatility and high shear stress deteriorate barrier phenotype in brain microvascular endothelium. *J. Cereb. Blood Flow Metab.* **37**, 2614–2625 (2017).
18. Brightman, M. W. & Reese, T. S. Junctions between intimately apposed cell membranes in the vertebrate brain. *J. Cell Biol.* **40**, 648–677 (1969).
19. Laksitorini, M. D., Yathindranath, V., Xiong, W., Hombach-Klonisch, S. & Miller, D. W. Modulation of Wnt/β-catenin signaling promotes blood–brain barrier phenotype in cultured brain endothelial cells. *Sci. Rep.* **9**, 19718 (2019).
20. Daneman, R. et al. Wnt/beta-catenin signaling is required for CNS, but not non-CNS, angiogenesis. *Proc. Natl Acad. Sci. USA* **106**, 641–646 (2009).
21. Stenman, J. M. et al. Canonical Wnt signaling regulates organ-specific assembly and differentiation of CNS vasculature. *Science* **322**, 1247–1250 (2008).
22. Ben-Zvi, A. et al. Mfsd2a is critical for the formation and function of the blood–brain barrier. *Nature* **509**, 507–511 (2014).
23. Kutuzov, N., Flyvbjerg, H. & Lauritzen, M. Contributions of the glycocalyx, endothelium, and extravascular compartment to the blood–brain Barrier. *Proc. Natl Acad. Sci. USA* **115**, E9429–E9438 (2018).
24. Walter, F. R. et al. Surface charge, glycocalyx, and blood–brain barrier function. *Tissue Barriers* **9**, 1904773 (2021).
25. Jin, J. et al. The structure and function of the glycocalyx and its connection with blood–brain barrier. *Front. Cell Neurosci.* **15**, 739699 (2021).
26. De Bock, M. et al. Targeting gliovascular connexins prevents inflammatory blood–brain barrier leakage and astrogliosis. *JCI Insight* **7**, e135263 (2022).
27. De Bock, M. et al. Connexin channels provide a target to manipulate brain endothelial calcium dynamics and blood–brain barrier permeability. *J. Cereb. Blood Flow Metab.* **31**, 1942–1957 (2011).
28. Deracinois, B., Lenfant, A. M., Dehouck, M. P. & Flahaut, C. Tissue non-specific alkaline phosphatase (TNAP) in vessels of the brain. *Subcell. Biochem.* **76**, 125–151 (2015).
29. Hawkins, R. A., O'Kane, R. L., Simpson, I. A. & Viña, J. R. Structure of the blood–brain barrier and its role in the transport of amino acids. *J. Nutr.* **136**, 218S–226SS (2006).
30. Decleves, X. et al. Interplay of drug metabolizing CYP450 enzymes and ABC transporters in the blood–brain barrier. *Curr. Drug Metab.* **12**, 732–741 (2011).
31. Sato, R. et al. An atlas of the quantitative protein expression of anti-epileptic-drug transporters, metabolizing enzymes and tight junctions at the blood–brain barrier in epileptic patients. *Pharmaceutics* **13**, 2122 (2021).
32. Holm, A., Heumann, T. & Augustin, H. G. Microvascular mural cell organotypic heterogeneity and functional plasticity. *Trends Cell Biol.* **28**, 302–316 (2018).
33. Bergers, G. & Song, S. The role of pericytes in blood-vessel formation and maintenance. *Neuro Oncol.* **7**, 452–464 (2005).
34. Hartmann, D. A., Coelho-Santos, V. & Shih, A. Y. Pericyte control of blood flow across microvascular zones in the central nervous system. *Annu. Rev. Physiol.* **84**, 331 (2021).
35. Hellström, M., Kalén, M., Lindahl, P., Abramsson, A. & Betsholtz, C. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* **126**, 3047–3055 (1999).
36. Darland, D. C. & D'amore, P. A. TGF beta is required for the formation of capillary-like structures in three-dimensional cocultures of 10T1/2 and endothelial cells. *Angiogenesis* **4**, 11–20 (2001).
37. Rustenhoven, J. et al. TGF-beta1 regulates human brain pericyte inflammatory processes involved in neurovasculature function. *J. Neuroinflamm.* **13**, 37 (2016).
38. Schumacher, L. et al. TGF-beta modulates the integrity of the blood–brain barrier in vitro, and is associated with metabolic alterations in pericytes. *Biomedicines* **11**, 214 (2023).

39. Dohgu, S. et al. Brain pericytes contribute to the induction and up-regulation of blood–brain barrier functions through transforming growth factor-beta production. *Brain Res.* **1038**, 208–215 (2005).
40. Senatorov, V. V. et al. Blood–brain barrier dysfunction in aging induces hyperactivation of TGFβ signaling and chronic yet reversible neural dysfunction. *Sci. Transl. Med.* **11**, 8283 (2019).
41. Cuevas, P. et al. Pericyte endothelial gap junctions in human cerebral capillaries. *Anat. Embryol.* **170**, 155–159 (1984).
42. Geevarghese, A. & Herman, I. M. Pericyte–endothelial crosstalk: implications and opportunities for advanced cellular therapies. *Transl. Res.* **163**, 296–306 (2014).
43. Bennett, H. C. & Kim, Y. Pericytes across the lifetime in the central nervous system. *Front. Cell Neurosci.* **15**, 627291 (2021).
44. Cao, L., Zhou, Y., Chen, M., Li, L. & Zhang, W. Pericytes for therapeutic approaches to ischemic stroke. *Front. Neurosci.* **15**, 629297 (2021).
45. Bennett, H. C. et al. Aging drives cerebrovascular network remodeling and functional changes in the mouse brain. *Nat. Commun.* **15**, 629297 (2024).
46. Baldwin, K. T., Murai, K. K. & Khakh, B. S. Astrocyte morphology. *Trends Cell Biol.* **34**, 547–565 (2024).
47. Cibelli, A. et al. Cx43 carboxyl terminal domain determines AQP4 and Cx30 endfoot organization and blood brain barrier permeability. *Sci. Rep.* **11**, 24334 (2021).
48. Dermietzel, R., Hertzberg, E. L., Kassier, J. A. & Spray, D. C. Gap junctions between cultured astrocytes: immunocytochemical, molecular, and electrophysiological analysis. *J. Neurosci.* **11**, 1421–1432 (1991).
49. Menezes, M. J. et al. The extracellular matrix protein laminin α2 regulates the maturation and function of the blood-brain barrier. *J. Neurosci.* **34**, 15260–15280 (2014).
50. Tail, M. et al. Sonic Hedgehog reduces inflammatory response, decreases blood–spinal cord barrier permeability, and improves locomotor function recovery in an acute spinal cord injury rat model. *J. Inflamm.* **21**, 34 (2024).
51. Alvarez, J. I. et al. The Hedgehog pathway promotes blood–brain barrier integrity and CNS immune quiescence. *Science* **334**, 1727–1731 (2011).
52. Gavard, J., Patel, V. & Gutkind, J. S. Angiopoietin-1 prevents VEGF-induced endothelial permeability by sequestering src through mDia. *Dev. Cell* **14**, 25–36 (2008).
53. Lengfeld, J. E. et al. Endothelial Wnt/β-catenin signaling reduces immune cell infiltration in multiple sclerosis. *Proc. Natl Acad. Sci. USA* **114**, E1168–E1177 (2017).
54. Liebner, S. et al. Wnt/beta-catenin signaling controls development of the blood–brain barrier. *J. Cell Biol.* **183**, 409–417 (2008).
55. Bake, S., Okoreeh, A. K., Alaniz, R. C. & Sohrabji, F. Insulin-like growth factor (IGF)-I modulates endothelial blood–brain barrier function in ischemic middle-aged female rats. *Endocrinology* **157**, 61–69 (2016).
56. Yang, L. et al. Neurons enhance blood-brain barrier function via upregulating claudin-5 and VE-cadherin expression due to glial cell line-derived neurotrophic factor secretion. *eLife* **13**, RP96161 (2024).
57. Gulej, R. et al. Endothelial deficiency of insulin-like growth factor-1 receptor leads to blood–brain barrier disruption and accelerated endothelial senescence in mice, mimicking aspects of the brain aging phenotype. *Microcirculation* **31**, e12840 (2024).
58. Reuss, B., Dono, R. & Unsicker, K. Functions of fibroblast growth factor (FGF)-2 and FGF-5 in astroglial differentiation and blood–brain barrier permeability: evidence from mouse mutants. *J. Neurosci.* **23**, 6404–6412 (2003).
59. Jackson, R. J. et al. APOE4 derived from astrocytes leads to blood–brain barrier impairment. *Brain* **145**, 3582–3593 (2022).
60. Salman, M. M. et al. Emerging roles for dynamic aquaporin-4 subcellular relocalization in CNS water homeostasis. *Brain* **145**, 64–75 (2022).
61. Nagelhus, E. A. & Ottersen, O. P. Physiological roles of aquaporin-4 in brain. *Physiol. Rev.* **93**, 1543–1562 (2013).
62. Bloch, O. & Manley, G. T. The role of aquaporin-4 in cerebral water transport and edema. *Neurosurg. Focus.* **22**, E3 (2007).
63. Zhang, Y. et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* **34**, 11929–11947 (2014).
64. Sharma, K. et al. Cell type- and brain region-resolved mouse brain proteome. *Nat. Neurosci.* **18**, 1819–1831 (2015).
65. Anderson, M. A. et al. Astrocyte scar formation aids central nervous system axon regeneration. *Nature* **532**, 195–200 (2016).
66. Heithoff, B. P. et al. Astrocytes are necessary for blood–brain barrier maintenance in the adult mouse brain. *Glia* **69**, 436–472 (2021).
67. Kabba, J. A. et al. Microglia: housekeeper of the central nervous system. *Cell Mol. Neurobiol.* **38**, 53–71 (2018).
68. Crapser, J. D., Arreola, M. A., Tsourmas, K. I. & Green, K. N. Microglia as hackers of the matrix: sculpting synapses and the extracellular space. *Cell Mol. Immunol.* **18**, 2472–2488 (2021).
69. Akiyoshi, R. et al. Microglia enhance synapse activity to promote local network synchronization. *eNeuro* **5**, ENEURO.0088-18.2018 (2018).
70. Basilico, B. et al. Microglia shape presynaptic properties at developing glutamatergic synapses. *Glia* **67**, 53–67 (2019).
71. Badimon, A. et al. Negative feedback control of neuronal activity by microglia. *Nature* **586**, 417–423 (2020).
72. Diaz-Aparicio, I. et al. Microglia actively remodel adult hippocampal neurogenesis through the phagocytosis secretome. *J. Neurosci.* **40**, 1453–1482 (2020).
73. Sierra, A. et al. Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* **7**, 483–495 (2010).
74. Mondo, E. et al. A developmental analysis of juxtavascular microglia dynamics and interactions with the vasculature. *J. Neurosci.* **40**, 6503–6521 (2020).
75. Haruwaka, K. et al. Dual microglia effects on blood–brain barrier permeability induced by systemic inflammation. *Nat. Commun.* **10**, 5816 (2019).
76. Shigemoto-Mogami, Y., Hoshikawa, K. & Sato, K. Activated microglia disrupt the blood–brain barrier and induce chemokines and cytokines in a rat in vitro model. *Front. Cell Neurosci.* **12**, 494 (2018).
77. Wang, Y. et al. Interleukin-1β induces blood–brain barrier disruption by downregulating sonic hedgehog in astrocytes. *PLoS ONE* **9**, e110024 (2014).
78. Denes, A. et al. Endothelial cells and macrophages as allies in the healthy and diseased brain. *Acta Neuropathol.* **147**, 38 (2024).
79. Lin, R. et al. Depleting parenchymal border macrophages alleviates cerebral edema and neuroinflammation following status epilepticus. *J. Transl. Med.* **22**, 1094 (2024).
80. Nagy, Z., Pappius, H. M., Mathieson, G. & Hüttner, I. Opening of tight junctions in cerebral endothelium. I. Effect of hyperosmolar mannitol infused through the internal carotid artery. *J. Comp. Neurol.* **185**, 569–578 (1979).
81. Reese, T. S. & Karnovsky, M. J. Fine structural localization of a blood–brain barrier to exogenous peroxidase. *J. Cell Biol.* **34**, 207–217 (1967).
82. Luissint, A. C., Artus, C., Glacial, F., Ganeshamoorthy, K. & Couraud, P. O. Tight junctions at the blood–brain barrier: physiological architecture and disease-associated dysregulation. *Fluids Barriers CNS* **9**, 23 (2012).
83. Furuse, M. et al. Occludin: a novel integral membrane protein localizing at tight junctions. *J. Cell Biol.* **123**, 1777–1788 (1993).
84. Furuse, M., Fujita, K., Hiiragi, T., Fujimoto, K. & Tsukita, S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J. Cell Biol.* **141**, 1539–1550 (1998).
85. Kooij, G., Van Horssen, J. & De Vries, E. in *The Blood–Brain Barrier and its Microenvironment: Basic Physiology to Neurological Disease* (eds. de Vries, E. & Prat, A.) 47–69 (Taylor & Francis, 2005).
86. Bendriem, R. M., Singh, S., Aleem, A. A., Antonetti, D. A. & Ross, M. E. Tight junction protein occludin regulates progenitor self-renewal and survival in developing cortex. *eLife* **8**, e49376 (2019).
87. Kanayasu-Toyoda, T. et al. Occludin as a functional marker of vascular endothelial cells on tube-forming activity. *J. Cell Physiol.* **233**, 1700–1711 (2018).
88. Blasig, I. E. et al. Occludin protein family: oxidative stress and reducing conditions. *Antioxid. Redox Signal.* **15**, 1195–1219 (2011).
89. Balda, M. S. et al. Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *J. Cell Biol.* **134**, 1031–1049 (1996).
90. Wong, V. & Gumbiner, B. M. A synthetic peptide corresponding to the extracellular domain of occludin perturbs the tight junction permeability barrier. *J. Cell Biol.* **136**, 399–409 (1997).
91. Saitou, M. et al. Occludin-deficient embryonic stem cells can differentiate into polarized epithelial cells bearing tight junctions. *J. Cell Biol.* **141**, 397–408 (1998).
92. Greene, C., Hanley, N. & Campbell, M. Claudin-5: gatekeeper of neurological function. *Fluids Barriers CNS* **16**, 3 (2019).
93. Nitta, T. et al. Size-selective loosening of the blood–brain barrier in claudin-5-deficient mice. *J. Cell Biol.* **161**, 653–660 (2003).
94. Wolburg, H. et al. Localization of claudin-3 in tight junctions of the blood–brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme. *Acta Neuropathol.* **105**, 586–592 (2003).
95. Berndt, P. et al. Tight junction proteins at the blood–brain barrier: far more than claudin-5. *Cell Mol. Life Sci.* **76**, 1987–2002 (2019).
96. Castro Dias, M. et al. Claudin-3-deficient C57BL/6J mice display intact brain barriers. *Sci. Rep.* **9**, 203 (2019).
97. Marchetti, L. & Engelhardt, B. Immune cell trafficking across the blood–brain barrier in the absence and presence of neuroinflammation. *Vasc. Biol.* **2**, H1–H18 (2020).
98. Hartmann, C., Schwietzer, Y. A., Otani, T., Furuse, M. & Ebnet, K. Physiological functions of junctional adhesion molecules (JAMs) in tight junctions. *Biochim. Biophys. Acta Biomembr.* **1862**, 183299 (2020).
99. Bosveld, F., Wang, Z. & Bellaïche, Y. Tricellular junctions: a hot corner of epithelial biology. *Curr. Opin. Cell Biol.* **54**, 80–88 (2018).
100. Iwamoto, N., Higashi, T. & Furuse, M. Localization of angulin-1/LSR and tricellulin at tricellular contacts of brain and retinal endothelial cells in vivo. *Cell Struct. Funct.* **39**, 1–8 (2014).
101. Sohet, F. et al. LSR/angulin-1 is a tricellular tight junction protein involved in blood–brain barrier formation. *J. Cell Biol.* **208**, 703–711 (2015).
102. Dias, M. C. et al. Brain endothelial tricellular junctions as novel sites for T cell diapedesis across the blood–brain barrier. *J. Cell Sci.* **134**, jcs253880 (2021).
103. Giannotta, M., Trani, M. & Dejana, E. VE-cadherin and endothelial adherens junctions: active guardians of vascular integrity. *Dev. Cell* **26**, 441–454 (2013).
104. Azizi, P. M. et al. Clathrin-dependent entry and vesicle-mediated exocytosis define insulin transcytosis across microvascular endothelial cells. *Mol. Biol. Cell* **26**, 740–750 (2015).
105. Zhou, M. et al. Caveolae-mediated endothelial transcytosis across the blood–brain barrier in acute ischemic stroke. *J. Clin. Med.* **10**, 3795 (2021).
106. Zhang, W. et al. Differential expression of receptors mediating receptor-mediated transcytosis (RMT) in brain microvessels, brain parenchyma and peripheral tissues of the mouse and the human. *Fluids Barriers CNS* **17**, 47 (2020).

107. Haqqani, A. S., Bélanger, K. & Stanimirovic, D. B. Receptor-mediated transcytosis for brain delivery of therapeutics: receptor classes and criteria. *Front. Drug Deliv.* **4**, 1360302 (2024).
108. Pemberton, S., Galindo, D. C., Schwartz, M. W., Banks, W. A. & Rhea, E. M. Endocytosis of insulin at the blood–brain barrier. *Front. Drug Deliv.* **2**, 1062366 (2022).
109. Barr, V. A., Lane, K. & Taylor, S. I. Subcellular localization and internalization of the four human leptin receptor isoforms. *J. Biol. Chem.* **274**, 21416–21424 (1999).
110. Barrett, G. L., Trieu, J. & Naim, T. The identification of leptin-derived peptides that are taken up by the brain. *Regul. Pept.* **155**, 55–61 (2009).
111. Fishman, J. B., Rubin, J. B., Handrahan, J. V., Connor, J. R. & Fine, R. E. Receptor-mediated transcytosis of transferrin across the blood–brain barrier. *J. Neurosci. Res.* **18**, 299–304 (1987).
112. Pflanzner, T. et al. LRP1 mediates bidirectional transcytosis of amyloid-β across the blood–brain barrier. *Neurobiol. Aging* **32**, 2323.e1–2323.e11 (2011).
113. Kiss, R. S. et al. The lipoprotein receptor-related protein-1 (LRP) adapter protein GULP mediates trafficking of the LRP ligand prosaposin, leading to sphingolipid and free cholesterol accumulation in late endosomes and impaired efflux. *J. Biol. Chem.* **281**, 12081–12092 (2006).
114. Zhao, S., Chai, X. & Frotscher, M. Balance between neurogenesis and gliogenesis in the adult hippocampus: role for reelin. *Dev. Neurosci.* **29**, 84–90 (2007).
115. Bock, H. H. et al. Phosphatidylinositol 3-kinase interacts with the adaptor protein Dab1 in response to Reelin signaling and is required for normal cortical lamination. *J. Biol. Chem.* **278**, 38772–38779 (2003).
116. Tissir, F. & Goffinet, A. M. Reelin and brain development. *Nat. Rev. Neurosci.* **4**, 496–505 (2003).
117. Coleman, J. A. & Molday, R. S. Critical role of the beta-subunit CDC50A in the stable expression, assembly, subcellular localization, and lipid transport activity of the P4-ATPase ATP8A2. *J. Biol. Chem.* **286**, 17205–17216 (2011).
118. Sebastian, T. T., Baldridge, R. D., Xu, P. & Graham, T. R. Phospholipid flippases: building asymmetric membranes and transport vesicles. *Biochim. Biophys. Acta* **1821**, 1068–1077 (2012).
119. Zhu, X., Jin, K., Huang, Y. & Pang, Z. in *Brain Targeted Drug Delivery System* (eds Gao, H. & Gao, X.) 159–183 (Academic, 2019).
120. Hervé, F., Ghinea, N. & Scherrmann, J. M. CNS delivery via adsorptive transcytosis. *AAPS J.* **10**, 455–472 (2008).
121. Palade, G. E. Fine structure of blood capillaries. *J. Appl. Phys.* **24**, 1424–1448 (1953).
122. YAMADA, E. The fine structure of the gall bladder epithelium of the mouse. *J. Biophys. Biochem. Cytol.* **1**, 445–458 (1955).
123. Parton, R. G. & Del Pozo, M. A. Caveolae as plasma membrane sensors, protectors and organizers. *Nat. Rev. Mol. Cell Biol.* **14**, 98–112 (2013).
124. Smith, K. R. & Borchardt, R. T. Permeability and mechanism of albumin, cationized albumin, and glycosylated albumin transcellular transport across monolayers of cultured bovine brain capillary endothelial cells. *Pharm. Res.* **6**, 466–473 (1989).
125. Schnitzer, J. E. gp60 is an albumin-binding glycoprotein expressed by continuous endothelium involved in albumin transcytosis. *Am. J. Physiol.* **262**, H246–H254 (1992).
126. Dehouck, B. et al. A new function for the LDL receptor: transcytosis of LDL across the blood–brain barrier. *J. Cell Biol.* **138**, 877–889 (1997).
127. Deane, R. et al. RAGE mediates amyloid-beta peptide transport across the blood–brain barrier and accumulation in brain. *Nat. Med.* **9**, 907–913 (2003).
128. Trevino, T. N. & Lutz, S. E. Matrix proteins plug a hole: how pericytes suppress blood–brain barrier transcytosis. *Neuron* **110**, 1601–1603 (2022).
129. Chow, B. W. & Gu, C. Gradual suppression of transcytosis governs functional blood–retinal barrier formation. *Neuron* **93**, 1325–1333.e3 (2017).
130. Ayloo, S. et al. Pericyte-to-endothelial cell signaling via vitronectin-integrin regulates blood–CNS barrier. *Neuron* **110**, 1641–1655.e6 (2022).
131. Wang, Z. et al. Wnt signaling activates MFSD2A to suppress vascular endothelial transcytosis and maintain blood–retinal barrier. *Sci. Adv.* **6**, eaba7457 (2020).
132. Andreone, B. J. et al. Blood–brain barrier permeability is regulated by lipid transport-dependent suppression of caveolae-mediated transcytosis. *Neuron* **94**, 581–594.e5 (2017).
133. Hediger, M. A., Clémençon, B., Burrier, R. E. & Bruford, E. A. The ABCs of membrane transporters in health and disease. *Mol. Asp. Med.* **34**, 338–352 (2013).
134. Mueckler, M. & Thorens, B. The SLC2 (GLUT) family of membrane transporters. *Mol. Asp. Med.* **34**, 121–138 (2013).
135. Striano, P. et al. GLUT1 mutations are a rare cause of familial idiopathic generalized epilepsy. *Neurology* **78**, 557–562 (2012).
136. Koch, H. & Weber, Y. G. The glucose transporter type 1 (Glut1) syndromes. *Epilepsy Behav.* **91**, 90–93 (2019).
137. Corradini, M. et al. GLUT1DS focus on dysarthria. *Eur. J. Paediatr. Neurol.* **51**, 62–70 (2024).
138. Zaragozá, R. Transport of amino acids across the blood–brain barrier. *Front. Physiol.* **11**, 973 (2020).
139. Boado, R. J., Li, J. Y., Nagaya, M., Zhang, C. & Pardridge, W. M. Selective expression of the large neutral amino acid transporter at the blood–brain barrier. *Proc. Natl Acad. Sci. USA* **96**, 12079–12084 (1999).
140. Devés, R. & Boyd, C. A. R. Transporters for cationic amino acids in animal cells: discovery, structure, and function. *Physiol. Rev.* **78**, 487–545 (1998).
141. Stoll, J., Wadhwani, K. C. & Smith, Q. R. Identification of the cationic amino acid transporter (System y+) of the rat blood–brain barrier. *J. Neurochem.* **60**, 1956–1959 (1993).
142. O'Kane, R. L. et al. Cationic amino acid transport across the blood–brain barrier is mediated exclusively by system y+. *Am. J. Physiol. Endocrinol. Metab.* **291**, E412–E419 (2006).
143. Nałęcz, K. A. Solute carriers in the blood–brain barrier: safety in abundance. *Neurochem. Res.* **42**, 795–809 (2017).
144. Jen, J. C., Wan, J., Palos, T. P., Howard, B. D. & Baloh, R. W. Mutation in the glutamate transporter EAAT1 causes episodic ataxia, hemiplegia, and seizures. *Neurology* **65**, 529–534 (2005).
145. Alam, A. & Locher, K. P. Structure and mechanism of human ABC transporters. *Annu. Rev. Biophys.* **52**, 275–300 (2023).
146. Dean, M., Hamon, Y. & Chimini, G. The human ATP-binding cassette (ABC) transporter superfamily. *J. Lipid Res.* **42**, 1007–1017 (2001).
147. Chen, J., Zhang, X., Kusumo, H., Costa, L. G. & Guizzetti, M. Cholesterol efflux is differentially regulated in neurons and astrocytes: implications for brain cholesterol homeostasis. *Biochim. Biophys. Acta* **1831**, 263–275 (2013).
148. Bauer, M., Tournier, N. & Langer, O. Imaging P-glycoprotein function at the blood–brain barrier as a determinant of the variability in response to central nervous system drugs. *Clin. Pharmacol. Ther.* **105**, 1061–1064 (2019).
149. Tai, L. M., Loughlin, A. J., Male, D. K. & Romero, I. A. P-glycoprotein and breast cancer resistance protein restrict apical-to-basolateral permeability of human brain endothelium to amyloid-beta. *J. Cereb. Blood Flow Metab.* **29**, 1079–1083 (2009).
150. Robey, R. W. et al. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat. Rev. Cancer* **18**, 452–464 (2018).
151. Schulz, J. A., Hartz, A. M. S. & Bauer, B. ABCB1 and ABCG2 regulation at the blood–brain barrier: potential new targets to improve brain drug delivery. *Pharmacol. Rev.* **75**, 815–853 (2023).
152. Sadeghian, H. et al. Spreading depolarizations trigger caveolin-1-dependent endothelial transcytosis. *Ann. Neurol.* **84**, 409–423 (2018).
153. Prager, O. et al. Seizure-induced microvascular injury is associated with impaired neurovascular coupling and blood–brain barrier dysfunction. *Epilepsia* **60**, 322–336 (2019).
154. Swissa, E. et al. Cortical plasticity is associated with blood-brain barrier modulation. *eLife* **12**, RP89611 (2024).
155. Vazana, U. et al. TMS-induced controlled BBB opening: preclinical characterization and implications for treatment of brain cancer. *Pharmaceutics* **12**, 1–16 (2020).
156. Vazana, U. et al. Glutamate-mediated blood–brain barrier opening: implications for neuroprotection and drug delivery. *J. Neurosci.* **36**, 7727–7739 (2016).
157. Liu, X., Chi, O. Z. & Weiss, H. R. Effects of metabotropic glutamate receptor stimulation on blood–brain barrier permeability during focal cerebral ischemia. *Neurochem. Res.* **29**, 1857–1862 (2004).
158. András, I. E. et al. The NMDA and AMPA/KA receptors are involved in glutamate-induced alterations of occludin expression and phosphorylation in brain endothelial cells. *J. Cereb. Blood Flow Metab.* **27**, 1431–1443 (2007).
159. Pulido, R. S. et al. Neuronal activity regulates blood–brain barrier efflux transport through endothelial circadian genes. *Neuron* **108**, 937–952.e7 (2020).
160. Zhang, S. L. et al. A circadian clock regulates efflux by the blood–brain barrier in mice and human cells. *Nat. Commun.* **12**, 617 (2021).
161. Pan, W., Cornélissen, G., Halberg, F. & Kastin, A. J. Selected contribution: circadian rhythm of tumor necrosis factor-alpha uptake into mouse spinal cord. *J. Appl. Physiol.* **92**, 1357–1362 (2002).
162. Pan, W. & Kastin, A. J. Diurnal variation of leptin entry from blood to brain involving partial saturation of the transport system. *Life Sci.* **68**, 2705–2714 (2001).
163. Kress, G. J. et al. Regulation of amyloid-β dynamics and pathology by the circadian clock. *J. Exp. Med.* **215**, 1059–1068 (2018).
164. Nakazato, R. et al. Disruption of bmal1 impairs blood-brain barrier integrity via pericyte dysfunction. *J. Neurosci.* **37**, 10052–10062 (2017).
165. Mastrullo, V. et al. Pericytes' circadian clock affects endothelial cells' synchronization and angiogenesis in a 3D tissue engineered scaffold. *Front. Pharmacol.* **13**, 867070 (2022).
166. Zhang, S. L., Yue, Z., Arnold, D. M., Artiushin, G. & Sehgal, A. A circadian clock in the blood–brain barrier regulates xenobiotic efflux. *Cell* **173**, 130–139.e10 (2018).
167. Hablitz, L. M. et al. Circadian control of brain glymphatic and lymphatic fluid flow. *Nat. Commun.* **11**, 4411 (2020).
168. Yao, D. et al. Melatonin alleviates depression-like behaviors and cognitive dysfunction in mice by regulating the circadian rhythm of AQP4 polarization. *Transl. Psychiatry* **13**, 310 (2023).
169. Huang, H. et al. Phosphorylation of AQP4 by LRRK2 R1441G impairs glymphatic clearance of IFNγ and aggravates dopaminergic neurodegeneration. *npj Parkinsons Dis.* **10**, 31 (2024).
170. Pati, R. et al. The readthrough isoform AQP4ex is constitutively phosphorylated in the perivascular astrocyte endfeet of human brain. *Biomolecules* **12**, 633 (2022).
171. Cuddapah, V. A., Zhang, S. L. & Sehgal, A. Regulation of the blood–brain barrier by circadian rhythms and sleep. *Trends Neurosci.* **42**, 500–510 (2019).
172. Artiushin, G., Zhang, S. L., Tricoire, H. & Sehgal, A. Endocytosis at the *Drosophila* blood–brain barrier as a function for sleep. *eLife* **7**, e43326 (2018).
173. Skapetze, L. et al. Rhythms in barriers and fluids: circadian clock regulation in the aging neurovascular unit. *Neurobiol. Dis.* **181**, 106120 (2023).
174. Park, M., Levine, H. & Toborek, M. Exercise protects against methamphetamine-induced aberrant neurogenesis. *Sci. Rep.* **6**, 34111 (2016).
175. Bailey, D. M. et al. Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood–brain barrier leakage. *Exp. Physiol.* **96**, 1196–1207 (2011).
176. Małkiewicz, M. A. et al. Blood–brain barrier permeability and physical exercise. *J. Neuroinflamm.* **16**, 15 (2019).

177. Vecchio, L. M. et al. The neuroprotective effects of exercise: maintaining a healthy brain throughout aging. *Brain plasticity* **4**, 17–52 (2018).
178. Paton, S. E. J. et al. Environmental enrichment and physical exercise prevent stress-induced behavioral and blood–brain barrier alterations via Fgf2. *Biol. Psychiatry* **97** (Suppl.), S20–S21 (2025).
179. Chasseigneaux, S. et al. Fasting upregulates the monocarboxylate transporter MCT1 at the rat blood–brain barrier through PPAR δ activation. *Fluids Barriers CNS* **21**, 33 (2024).
180. Ito, S., Yanai, M., Yamaguchi, S., Couraud, P. O. & Ohtsuki, S. Regulation of tight-junction integrity by insulin in an in vitro model of human blood–brain barrier. *J. Pharm. Sci.* **106**, 2599–2605 (2017).
181. Wang, Z., Tang, X., Swaminathan, S. K., Kandimalla, K. K. & Kalari, K. R. Mapping the dynamics of insulin-responsive pathways in the blood–brain barrier endothelium using time-series transcriptomics data. *npj Syst. Biol. Appl.* **8**, 29 (2022).
182. Konishi, M. et al. Endothelial insulin receptors differentially control insulin signaling kinetics in peripheral tissues and brain of mice. *Proc. Natl Acad. Sci. USA* **114**, E8478–E8487 (2017).
183. Bogush, M., Heldt, N. A. & Persidsky, Y. Blood–brain barrier injury in diabetes: unrecognized effects on brain and cognition. *J. Neuroimmune Pharmacol.* **12**, 593–601 (2017).
184. Li, X., Cai, Y., Zhang, Z. & Zhou, J. Glial and vascular cell regulation of the blood–brain barrier in diabetes. *Diabetes Metab. J.* **46**, 222–238 (2022).
185. Feng, Z., Fang, C., Ma, Y. & Chang, J. Obesity-induced blood–brain barrier dysfunction: phenotypes and mechanisms. *J. Neuroinflamm.* **21**, 110 (2024).
186. Cheng, Y. et al. TNFα disrupts blood–brain barrier integrity to maintain prolonged depressive-like behavior in mice. *Brain Behav. Immun.* **69**, 556 (2018).
187. Salvador, E., Shityakov, S. & Förster, C. Glucocorticoids and endothelial cell barrier function. *Cell Tissue Res.* **355**, 597–605 (2014).
188. Lau, K., Kotzur, R. & Richter, F. Blood–brain barrier alterations and their impact on Parkinson's disease pathogenesis and therapy. *Transl. Neurodegener.* **13**, 37 (2024).
189. Tomkins, O. et al. Blood–brain barrier disruption results in delayed functional and structural alterations in the rat neocortex. *Neurobiol. Dis.* **25**, 367–377 (2007).
190. Cacheaux, L. P. et al. Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis. *J. Neurosci.* **29**, 8927–8935 (2009).
191. Tan, L. Y. et al. Emergence of the brain-border immune niches and their contribution to the development of neurodegenerative diseases. *Front. Immunol.* **15**, 1380063 (2024).
192. Shi, S. M. et al. Glycocalyx dysregulation impairs blood–brain barrier in ageing and disease. *Nature* **639**, 985–994 (2025).
193. Yang, A. C. et al. Physiological blood–brain transport is impaired with age by a shift in transcytosis. *Nature* **583**, 425–430 (2020).
194. Preininger, M. K. & Kaufer, D. Blood–brain barrier dysfunction and astrocyte senescence as reciprocal drivers of neuropathology in aging. *Int. J. Mol. Sci.* **23**, 6217 (2022).
195. Na, W., Lee, J. Y., Kim, W. S., Yune, T. Y. & Ju, B. G. 17β-estradiol ameliorates tight junction disruption via repression of MMP transcription. *Mol. Endocrinol.* **29**, 1347–1361 (2015).
196. Maggioli, E. et al. Estrogen protects the blood–brain barrier from inflammation-induced disruption and increased lymphocyte trafficking. *Brain Behav. Immun.* **51**, 212–222 (2016).
197. Li, Y. et al. N-myc downstream-regulated gene 2, a novel estrogen-targeted gene, is involved in the regulation of Na^+/K^+-ATPase. *J. Biol. Chem.* **286**, 32289–32299 (2011).
198. Atallah, A., Mhaouty-Kodja, S. & Grange-Messent, V. Chronic depletion of gonadal testosterone leads to blood–brain barrier dysfunction and inflammation in male mice. *J. Cereb. Blood Flow Metab.* **37**, 3161–3175 (2017).
199. Li, Z. et al. Progesterone increases circulating endothelial progenitor cells and induces neural regeneration after traumatic brain injury in aged rats. *J. Neurotrauma* **29**, 343–353 (2012).
200. Lei, Z. M., Rao, C. V., Kornyei, J. L., Licht, P. & Hiatt, E. S. Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. *Endocrinology* **132**, 2262–2270 (1993).
201. Wilson, A. C. et al. Reproductive hormones regulate the selective permeability of the blood–brain barrier. *Biochim. Biophys. Acta Mol. Basis Dis.* **1782**, 401–407 (2008).
202. Chechko, N. et al. The expectant brain-pregnancy leads to changes in brain morphology in the early postpartum period. *Cereb. Cortex* **32**, 4025–4038 (2022).
203. Cipolla, M. J., Sweet, J. G. & Chan, S. L. Cerebral vascular adaptation to pregnancy and its role in the neurological complications of eclampsia. *J. Appl. Physiol.* **110**, 329–339 (2011).
204. Zhou, Z. et al. Progesterone decreases gut permeability through upregulating occludin expression in primary human gut tissues and Caco-2 cells. *Sci. Rep.* **9**, 8367 (2019).
205. Si, D. et al. Progesterone protects blood–brain barrier function and improves neurological outcome following traumatic brain injury in rats. *Exp. Ther. Med.* **8**, 1010–1014 (2014).
206. Bergman, L. et al. Evidence of neuroinflammation and blood–brain barrier disruption in women with preeclampsia and eclampsia. *Cells* **10**, 3045 (2021).
207. Somjen, G. G. The influence of ion concentrations on neuron functions. *Ions Brain* https://doi.org/10.1093/OSO/9780195151718.003.0004 (2004).
208. Harik, S. I. Blood–brain barrier sodium/potassium pump: modulation by central noradrenergic innervation. *Proc. Natl Acad. Sci. USA* **83**, 4067–4070 (1986).
209. Harrington, M. G. et al. Capillary endothelial Na^+, K^+, ATPase transporter homeostasis and a new theory for migraine pathophysiology. *Headache J. Head Face Pain* **50**, 459–478 (2010).
210. O'Donnell, M. E. Blood–brain barrier Na transporters in ischemic stroke. *Adv. Pharmacol.* **71**, 113–146 (2014).
211. Salar, S. et al. Synaptic plasticity in area CA1 of rat hippocampal slices following intraventricular application of albumin. *Neurobiol. Dis.* **91**, 155–165 (2016).
212. Weissberg, I. et al. Albumin induces excitatory synaptogenesis through astrocytic TGF-β/ALK5 signaling in a model of acquired epilepsy following blood–brain barrier dysfunction. *Neurobiol. Dis.* **78**, 115–125 (2015).
213. Kim, S. Y. et al. TGFβ signaling is associated with changes in inflammatory gene expression and perineuronal net degradation around inhibitory neurons following various neurological insults. *Sci. Rep.* **7**, 7711 (2017).
214. Werther, G. A. et al. Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry. *Endocrinology* **121**, 1562–1570 (1987).
215. Banks, W. A., Jaspan, J. B., Huang, W. & Kastin, A. J. Transport of insulin across the blood–brain barrier: saturability at euglycemic doses of insulin. *Peptides* **18**, 1423–1429 (1997).
216. Kleinridders, A., Ferris, H. A., Cai, W. & Kahn, C. R. Insulin action in brain regulates systemic metabolism and brain function. *Diabetes* **63**, 2232–2243 (2014).
217. Shypshyna, M., Kolesnyk, O., Fedulova, S. & Veselovsky, N. Insulin modulates the paired-pulse plasticity at glutamatergic synapses of hippocampal neurons under hypoinsulinemia. *Front. Cell Neurosci.* **17**, 1132325 (2023).
218. Pomytkin, I. et al. Insulin receptor in the brain: mechanisms of activation and the role in the CNS pathology and treatment. *CNS Neurosci. Ther.* **24**, 763–774 (2018).
219. Bingham, E. M. et al. The role of insulin in human brain glucose metabolism: an 18fluoro-deoxyglucose positron emission tomography study. *Diabetes* **51**, 3384–3390 (2002).
220. Wardelmann, K. et al. Insulin action in the brain regulates mitochondrial stress responses and reduces diet-induced weight gain. *Mol. Metab.* **21**, 68–81 (2019).
221. Brabazon, F. et al. Intranasal insulin treatment of an experimental model of moderate traumatic brain injury. *J. Cereb. Blood Flow Metab.* **37**, 3203–3218 (2017).
222. Zou, X. H., Sun, L. H., Yang, W., Li, B. J. & Cui, R. J. Potential role of insulin on the pathogenesis of depression. *Cell Prolif.* **53**, e12806 (2020).
223. McNay, E. C. & Recknagel, A. K. Brain insulin signaling: a key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes. *Neurobiol. Learn. Mem.* **96**, 432–442 (2011).
224. Zhao, W. Q. & Alkon, D. L. Role of insulin and insulin receptor in learning and memory. *Mol. Cell. Endocrinol.* **177**, 125–134 (2001).
225. Ryan, J. P., Karim, H. T., Aizenstein, H. J., Helbling, N. L. & Toledo, F. G. S. Insulin sensitivity predicts brain network connectivity following a meal. *NeuroImage* **171**, 268–276 (2018).
226. Spinelli, M., Fusco, S. & Grassi, C. Brain insulin resistance impairs hippocampal plasticity. *Vitam. Horm.* **114**, 281–306 (2020).
227. Willmann, C. et al. Insulin sensitivity predicts cognitive decline in individuals with prediabetes. *BMJ Open Diabetes Res. Care* **8**, e001741 (2020).
228. Kamintsky, L. et al. Blood–brain barrier imaging as a potential biomarker for bipolar disorder progression. *Neuroimage Clin.* **26**, 102049 (2020).
229. van Hameren, G. et al. From spreading depolarization to blood–brain barrier dysfunction: navigating traumatic brain injury for novel diagnosis and therapy. *Nat. Rev. Neurol.* **20**, 408–425 (2024).
230. Ha, I. H. et al. Regional differences in blood–brain barrier permeability in cognitively normal elderly subjects: a dynamic contrast-enhanced MRI-based study. *Korean J. Radiol.* **22**, 1152–1162 (2021).
231. Hsu, J. L. et al. Magnetic resonance images implicate that glymphatic alterations mediate cognitive dysfunction in Alzheimer disease. *Ann. Neurol.* **93**, 164–174 (2023).
232. Harrison, I. F. et al. Impaired glymphatic function and clearance of tau in an Alzheimer's disease model. *Brain* **143**, 2576–2593 (2020).
233. Iliff, J. J. et al. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *J. Clin. Invest.* **123**, 1299–1309 (2013).
234. Hladky, S. B. & Barrand, M. A. The glymphatic hypothesis: the theory and the evidence. *Fluids Barriers CNS* **19**, 9 (2022).
235. Verheggen, I. C. M., Van Boxtel, M. P. J., Verhey, F. R. J., Jansen, J. F. A. & Backes, W. H. Interaction between blood–brain barrier and glymphatic system in solute clearance. *Neurosci. Biobehav. Rev.* **90**, 26–33 (2018).
236. Menard, C. et al. Social stress induces neurovascular pathology promoting depression. *Nat. Neurosci.* **20**, 1752–1760 (2017).
237. Dion-Albert, L. et al. Vascular and blood–brain barrier-related changes underlie stress responses and resilience in female mice and depression in human tissue. *Nat. Commun.* **13**, 164 (2022).
238. Daneman, R., Zhou, L., Kebede, A. A. & Barres, B. A. Pericytes are required for blood–brain barrier integrity during embryogenesis. *Nature* **468**, 562–566 (2010).
239. Liu, S., Agalliu, D., Yu, C. & Fisher, M. The role of pericytes in blood–brain barrier function and stroke. *Curr. Pharm. Des.* **18**, 3653–3662 (2012).
240. Rangel-Gomez, M. et al. Neuron–glial interactions: implications for plasticity, behavior, and cognition. *J. Neurosci.* **44**, e1231242024 (2024).
241. Haydon, P. G. & Carmignoto, G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol. Rev.* **86**, 1009–1031 (2006).
242. Le, A. D. et al. Astrocyte modulation of synaptic plasticity mediated by activity-dependent sonic hedgehog signaling. *J. Neurosci.* **45**, e1336242025 (2025).
243. Wu, Y., Dissing-Olesen, L., MacVicar, B. A. & Stevens, B. Microglia: dynamic mediators of synapse development and plasticity. *Trends Immunol.* **36**, 605–613 (2015).
244. Storck, S. E. et al. Endothelial LRP1 transports amyloid-β(1–42) across the blood–brain barrier. *J. Clin. Invest.* **126**, 123–136 (2016).
245. David, Y. et al. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *J. Neurosci.* **29**, 10588–10599 (2009).
246. Zhang, Y. & Yang, X. The roles of TGF-β signaling in cerebrovascular diseases. *Front. Cell Dev. Biol.* **8**, 567682 (2020).
247. Arnold, T. D. et al. Excessive vascular sprouting underlies cerebral hemorrhage in mice lacking αVβ8-TGFβ signaling in the brain. *Development* **141**, 4489–4499 (2014).

248. Reyahi, A. et al. Foxf2 is required for brain pericyte differentiation and development and maintenance of the blood–brain barrier. *Dev. Cell* **34**, 19–32 (2015).
249. Li, F. et al. Endothelial Smad4 maintains cerebrovascular integrity by activating N-cadherin through cooperation with Notch. *Dev. Cell* **20**, 291–302 (2011).
250. Hori, S., Ohtsuki, S., Hosoya, K. I., Nakashima, E. & Terasaki, T. A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro. *J. Neurochem.* **89**, 503–513 (2004).
251. Iadecola, C. The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. *Neuron* **96**, 17–42 (2017).
252. Hamilton, N. B., Attwell, D. & Hall, C. N. Pericyte-mediated regulation of capillary diameter: a component of neurovascular coupling in health and disease. *Front. Neuroenerget.* **2**, 1 (2010).
253. Hall, C. N. et al. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* **508**, 55–60 (2014).
254. Hariharan, A., Robertson, C. D., Garcia, D. C. G. & Longden, T. A. Brain capillary pericytes are metabolic sentinels that control blood flow through a KATP channel-dependent energy switch. *Cell Rep.* **41**, 111872 (2022).
255. Peppiatt, C. M., Howarth, C., Mobbs, P. & Attwell, D. Bidirectional control of CNS capillary diameter by pericytes. *Nature* **443**, 700–704 (2006).
256. Cai, C. et al. Stimulation-induced increases in cerebral blood flow and local capillary vasoconstriction depend on conducted vascular responses. *Proc. Natl Acad. Sci. USA* **115**, E5796–E5804 (2018).
257. Hartmann, D. A. et al. Brain capillary pericytes exert a substantial but slow influence on blood flow. *Nat. Neurosci.* **24**, 633–645 (2021).
258. Yao, Y., Chen, Z. L., Norris, E. H. & Strickland, S. Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity. *Nat. Commun.* **5**, 3413 (2014).
259. Zhang, Z. G. et al. VEGF enhances angiogenesis and promotes blood–brain barrier leakage in the ischemic brain. *J. Clin. Invest.* **106**, 829–838 (2000).
260. Radisavljevic, Z., Avraham, H. & Avraham, S. Vascular endothelial growth factor up-regulates ICAM-1 expression via the phosphatidylinositol 3 OH-kinase/AKT/nitric oxide pathway and modulates migration of brain microvascular endothelial cells. *J. Biol. Chem.* **275**, 20770–20774 (2000).
261. Ranaivo, H. R., Hodge, J. N., Choi, N. & Wainwright, M. S. Albumin induces upregulation of matrix metalloproteinase-9 in astrocytes via MAPK and reactive oxygen species-dependent pathways. *J. Neuroinflamm.* **9**, 68 (2012).
262. Yang, Y., Estrada, E. Y., Thompson, J. F., Liu, W. & Rosenberg, G. A. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. *J. Cereb. Blood Flow Metab.* **27**, 697–709 (2007).
263. Kim, H. et al. Reactive astrocytes transduce inflammation in a blood–brain barrier model through a TNF-STAT3 signaling axis and secretion of alpha 1-antichymotrypsin. *Nat. Commun.* **13**, 6581 (2022).
264. Freeman, B. D. et al. Endothelin-1 mediates brain microvascular dysfunction leading to long-term cognitive impairment in a model of experimental cerebral malaria. *PLoS Pathog.* **12**, e1005477 (2016).
265. LeMaistre Stobart, J. L., Lu, L., Anderson, H. D. I., Mori, H. & Anderson, C. M. Astrocyte-induced cortical vasodilation is mediated by D-serine and endothelial nitric oxide synthase. *Proc. Natl Acad. Sci. USA* **110**, 3149–3154 (2013).
266. Janzer, R. C. & Raff, M. C. Astrocytes induce blood–brain barrier properties in endothelial cells. *Nature* **325**, 253–257 (1987).
267. Császár, E. et al. Microglia modulate blood flow, neurovascular coupling, and hypoperfusion via purinergic actions. *J. Exp. Med.* **219**, e20211071 (2022).
268. Bisht, K. et al. Capillary-associated microglia regulate vascular structure and function through PANX1–P2RY12 coupling in mice. *Nat. Commun.* **12**, 5289 (2021).
269. Mills, S. A. et al. Fractalkine-induced microglial vasoregulation occurs within the retina and is altered early in diabetic retinopathy. *Proc. Natl Acad. Sci. USA* **118**, e2112561118 (2021).
270. Brezzo, G., Simpson, J., Ameen-Ali, K. E., Berwick, J. & Martin, C. Acute effects of systemic inflammation upon the neuro-glial-vascular unit and cerebrovascular function. *Brain Behav. Immun. Health* **5**, 100074 (2020).
271. Hawkins, B. T., Lundeen, T. F., Norwood, K. M., Brooks, H. L. & Egleton, R. D. Increased blood–brain barrier permeability and altered tight junctions in experimental diabetes in the rat: contribution of hyperglycaemia and matrix metalloproteinases. *Diabetologia* **50**, 202–211 (2007).
272. Hu, B. et al. Disturbed neurovascular coupling in type 2 diabetes mellitus patients: evidence from a comprehensive fMRI analysis. *Neuroimage Clin.* **22**, 101802 (2019).
273. Starr, J. M. et al. Increased blood–brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging. *J. Neurol. Neurosurg. Psychiatry* **74**, 70–76 (2003).
274. Canna, A. et al. Neurovascular coupling in patients with type 2 diabetes mellitus. *Front. Aging Neurosci.* **14**, 976340 (2022).
275. Zhang, Y. et al. Neurovascular coupling alterations in type 2 diabetes: a 5-year longitudinal MRI study. *BMJ Open Diabetes Res. Care* **9**, e001433 (2021).

Author contributions

The authors all researched data for the article, provided substantial contributions to discussion of its content and the writing and reviewed and edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Neuroscience* thanks Mária A. Deli, Martin Lauritzen and Stefan Liebner for their contribution to the peer review of this work.

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